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Conflict of Interests: Multiple Signal Peptides With Diverging Goals

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ABSTRACT

Peptide signal sequences attached to or embedded into a core protein sequence control its cellular localization and several post-translational modifications. However, misleading or cumbersome results may be generated when expressing recombinant proteins with modified signal peptides or single domains of larger proteins. J. Cell. Biochem. 114: 510–513, 2013. © 2012 Wiley Periodicals, Inc.

KEY WORDS: LOCALIZATION SIGNALS; RECOMBINANT PROTEINS; ARTIFACTS

Peptide signal sequences attached to or embedded into a core protein sequence control its cellular localization and several post-translational modifications. The identification of peptide sequences conferring specific information has been exploited, for instance, to direct the accumulation of recombinant constructs in the non-physiological sub-cellular compartments or for the engineering of in vivo leader peptide-directed biosynthetic systems [Cardinale et al., 2004; Oman and van der Donk, 2010].

However, misleading or cumbersome results may be generated when expressing recombinant proteins with modified signal peptides. In Figure 1 we report an observation that we made when trying to express the recombinant Myc protein for purification purposes. It is well known that this is a nuclear protein and its recovery from cells is not feasible using mild buffers compatible with structure preservation. Although there are reports in which nuclear proteins were secreted after being modified by fusing a suitable signal peptide to the construct [Garnier et al., 1995], when the Myc sequence was subcloned in frame with a secretion signal peptide, the protein accumulated neither in the culture medium nor in the nucleus, but in the cytoplasm. This result was unpredicted but immediately identified as aberrant, since we knew where we would logically expect to find Myc. However, it could potentially lead us to completely wrong conclusions if we were testing the expression of an uncharacterized protein. Furthermore, some questions remain to be answered: Why secretion of nuclear proteins does work out for some of them and not for others? How can we evaluate the absolute and relative efficiency of signal peptides?

Indeed, the complexity of the in vivo equilibrium regulating, for instance, shuttling proteins should urge the study of the hierarchy of various signals present in any single sequence as to understand the consequences of the co-existence of signals with conflicting roles. Although we know that the de novo introduction of a new aberrant signal peptide to the nucleophosmin modifies its cellular localization and may lead to oncogenic transformation [Falini et al., 2006] as well as that a mutation inside the signal sequence prevents the secretion of preproparathyroid hormone in the endoplasmic reticulum thus determining its toxic accumulation [Datta et al., 2007], we do not have the understanding as to how exactly the mutations involving the signal peptides quantitatively alter their strength and consequently lead to a pathological condition. In other words, there are several examples in which signal peptide variants abrogating its functionality cause pathological sub-cellular localization [Szczesna-Skorupa et al., 1988; Kiraly et al., 2007], but it remains almost unknown whether mutations can modify the physiological state by simply tuning the strength of a signal peptide [Ronald et al., 2008] and, in doing so, alter slightly the sub-cellular equilibrium.

In practical terms, the missing knowledge may lead to misinterpretation of subcellular localization data and this effect may be amplified in the case of results obtained with "chopped proteins." It begs the question of whether researchers are careful enough when using separate domains of proteins to infer from their localization the localization of the complete protein that may host signal peptides capable of modifying the subcellular deliver. For instance, in a recent article [Park et al., 2012] the authors demonstrated that the sub-cellular localization of the NANOG transcription factor in COS-7 cells is regulated by two already known NLSs as well as by a (initially undetected) NES that they were

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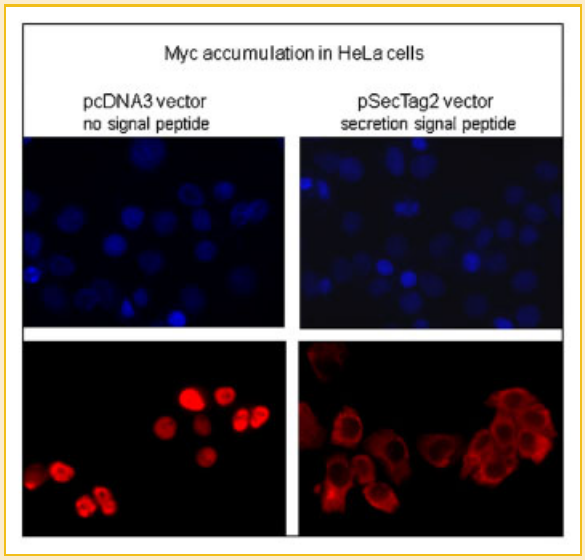

Fig. 1. Recombinant expression of Myc protein in HeLa cells. Myc was expressed either by using the pcDNA3 vector generating constructs without appended signal peptides or by using the pSecTag2 vector that enables the fusion of a secretion motive to the protein. HeLa cells were plated on slides pre-treated with 0.1% gelatin. Cells were transfected with 1 μg DNA/ml (pcDNA3.1-Myc or pSecTag2-Myc) and fixed in the presence of 4% paraformaldehyde. Anti-Myc primary antibody (9E10) was added at a concentration of 1 μg/ml. CY3-goat anti-mouse antibody, diluted 1:400 in PBS, was used as a secondary antibody. Slides were treated with DAPI (1:5,000 in PBS) for nuclear detection and assembled by using moviol.

finally able to characterize. In how many cases undetected signal sequence can determine the mislocalization of protein domains expressed independently? Consequently, should control data confirming the physiological significance of the localization experiments be mandatory to avoid accumulation of erroneous results in our archives?

Presently much is known about the consensus sequences that may act as signal peptides, but the reports dealing with parameters regulating their strength and activity modulation are very rare [Liu and Ganz, 1995; O'Sullivan et al., 2003; Holden et al., 2005]. To this end, a systematic investigation on the subject could reveal the mechanisms by which the efficacy of signal peptides can be tuned. In turn, this information would provide a rationale to predict how the modification of signal peptides incorporated into mature sequences in virtue of pathological or recombinant events might change their activity and, by modulating the trafficking rate of that protein between cell compartments, the cell metabolism.

Surprisingly, despite the effort devoted for identifying consensus sequences and designing precise algorithms for predicting functional classes of signal sequences relevant for protein trafficking, only few reports [Cioffi et al., 1989; Nothwehr and Gordon, 1989; Heger et al., 2001; Engelsma et al., 2004; Kutay and Güttinger, 2005; Geisberger et al., 2009] deal with quantitative and structural analyses aimed at evaluating how some residue modifications may drastically change the strength of a sequence intended for a specific job. Even more surprisingly, this research opportunity has been neglected even though the availability of supraphysiological nuclear export signals sufficient for tuning the protein shuttling allowed to elucidate critical mechanisms of interaction between the nuclear pore complex and CRM1 [Engelsma et al., 2004]. Since the steady-state localization of proteins shuttling between nucleus and cytoplasm depends on the rate of both fluxes, modifications of either NES or NLS will lead to altered physiological conditions, as well as chemical treatments may do so [Akaoumianaki et al., 2009].

The picture becomes even more complicated when proteins possess multiple signal peptides, namely several sequences that can be modified [Bolli et al., 2009; Rajamäki and Valkonen, 2009]. What does it happen when a further synthetic signal sequence is added or a domain of the protein is removed without considering the "sub-cellular information" contained in this portion? Is it meaningful to look for protein–protein interactions that occur in cell compartments that are precluded to a particular protein in physiological conditions without being aware of a potential artifact? Recently, Choudhary et al. [2009] showed that the Flt3 mutant kinase aberrantly phosphorylates inappropriate substrates because it is targeted to ER instead of the membrane, but what conclusions would have been inferred by less accurate researchers who would have limited their work to phosphorylation profile comparisons without analyzing the sub-cellular localization of wild type and mutant enzyme?

Whether we still do not know exactly what determines the strength of signal sequences, it has been demonstrated that some structural modifications can change it, as for instance in the case of net charge, hydrophobicity, phosphorylation, and disulfide bond formation [Beals et al., 1997; Kaffman et al., 1998; Kuge et al., 2001; Sorokin et al., 2007; Lee et al., 2011]. It is to be noted that these are on-off switch mechanisms rather than tuning systems, and similarly yes/no selection is also used for identifying canonical and unconventional signal sequences the abrogation of which alters the sub-cellular protein localization [Vissinga et al., 2009; Zaarour et al., 2009; Evangelisti et al., 2010; Katayama et al., 2010; Kovalenko et al., 2010]. Only few phosphorylation events have been described as sufficient for tuning the signal sequence strength of their transporter partners [Hübner et al., 1997; Briggs et al., 1998; Komeili and O'Shea, 1999], but we would expect that mutations resulting in a simple relative strength modification, namely altered trafficking rate, also happen. Are they too difficult to spot? Are odd data obtained with modified signal sequences missing because considered inconsistent and, consequently, not reported? Furthermore, we should consider that sometimes the real distribution of a specific protein in the different cell compartments is misunderstood just because of the lack of suitable reagents [Gruszka et al., 2012].

Recombinant protein expression is difficult to regulate [Carter and Reszka, 2002] and can perturb cell localization by means of several mechanisms that can interact with those mediated by signal peptides. Overexpression can saturate the physiological cell compartment and consequently protein can leak or be transported in other organelles [Arabi et al., 2003], misfolding can impair the structural recognition of translocation motives [Knodler et al., 2011] and crucial partners involved in trafficking can become limiting for correct delivery [Sundvall et al., 2012]. Finally, Kuusisto et al. [2012] have recently shown that SV40-induced cell transformation significantly modifies the nuclear localization-dependent nuclear

transport and correctly asked whether this standard technique is really reliable for producing model cell types since "transformed cells will almost certainly have significantly enhanced nuclear transport properties, which in turn may alter cell phenotype in various, yet-to-be-identified ways" and their use "may well not be suitable for many studies for which they are currently used as accepted practice."

If we consider that the artifacts generated by inducing heterologous protein expression and briefly listed in this comment can sum to those produced in live-cell imaging [Schnell et al., 2012], we could conclude that research should invest more resources in evaluating methodologies as well as in open-access repositories for collecting and comparing experimental data obtained using detailed protocols and minimal information guidelines [Nelson, 2009; Buckle et al., 2011]. Indeed, the results of two single methodological articles [Kuusisto et al., 2012; Schnell et al., 2012] might questions the data published in hundreds of other articles but this is the kind of contributions we need for better distinguishing robust data from putative artifacts. Specifically, we ask for more caution when dealing with protein domains that can be delivered in non-physiological compartments because of the addition of heterologous signal sequences or because native localization signals have been, consciously or not, removed.

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