Only functional localization is faithful localization

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Eukaryotic cells contain numerous compartments in which highly specialized functions are executed. The functional separation of key cellular processes, such as anabolic and catabolic pathways or the synthesis of RNA and proteins, is crucial for the precise coordination of the biological processes as well as for the extraordinary functional capacity and the high specialization of eukaryotic cells. Generation and maintenance of the subcellular compartmentalization requires accurate targeting of the individual constituents to these compartments in order to guarantee the proper cooperative function of proteins and RNA in the desired locations, yet not in other places of the cell. Elucidating the mechanisms underlying these trafficking and sorting processes has been a main focus of biological research over the past decades, and has identified hundreds of proteins functioning as targeting factors, trafficking chaperones, sorting components, and membrane translocons. Failure to properly localize RNA or proteins to their respective native locale leads to malfunction of the respective cellular compartment resulting in defective cell homeostasis, eventually even causing cell death. Mislocalization of cellular constituents frequently is associated with human disease. Most prominent cases are certain types of lysosomal storage diseases in which acidic hydrolases are mistargeted to the extracellular space leading to an accumulation of undigested material inside lysosomes. Likewise, a trafficking failure of a mutant version of cystic fibrosis transmembrane regulator CFTR retains the protein mainly in the secretory pathway leading to functional deficiency at its native location, i.e. the plasma membrane.

Numerous studies have revealed that a high number of cellular constituents are located in more than one compartment. This is the case during the dynamic shuttling of both RNA and proteins between the nucleus and cytosol during the various stages of gene expression and protein synthesis. Specialized export and import components assist the nucleo-cytoplasmic trafficking of, e.g., ribosomal proteins, transcription factors and gene regulatory components to assure proper synthesis and localization of cellular RNA and protein compounds. Up to a third of the cellular proteins are located in more than one compartment even without dynamic shuttling, and some of them appear to fulfill their particular function in both locations [1]. This suggests that the cell makes efficient use of a single protein’s function by targeting it to various locations. Examples of such proteins are aconitase and fumarase functioning in both mitochondria and the cytosolic-nuclear compartments; tRNA synthetases which aminoacylate their targets in both mitochondria and cytosol; cytochromes P450 and cytochrome b2 reductase which operate in both mitochondria and the endoplasmic reticulum. Numerous cases have been published where a particular protein is located predominantly in one compartment but is additionally present in small amounts at other places. This ‘eclipsed’ protein distribution immediately raises the question of whether the secondary, minor localization is functionally relevant or whether it is the result of inaccurate or inefficient targeting mechanisms [2]. Another problem concerning secondary protein localization may be a technical and not a biological one. To faithfully detect proteins, researchers are frequently forced to use overexpressed and/or epitope-tagged proteins. Both the surplus of proteins and alterations in the overall structure caused by tagging might result in erroneous subcellular targeting with no functional relevance. In light of these difficulties, the gold standard for determining the biological meaning of a potential secondary cellular domicile therefore is “functional localization”, i.e. the assignment of a particular function of the protein of interest in this locale. However, this approach is usually demanding, as functional assays might not be available or


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not specific for the considered subcellular compartment.

An interesting example is the cysteine desulfurase Nfs1
which is predominantly located in mitochondria where it
participates in cellular iron-sulfur (Fe/S) protein biogenesis
[3, 4]. Small amounts, however, have been detected out-
side mitochondria [5], yet its function does not appear to
be in cytosolic Fe/S protein biogenesis [6, 7]. While the
precise function of extra-mitochondrial Nfs1 remains a
matter of debate [8-10], yeast genetic studies have ele-
gantly shown that this version of Nfs1 is essential for cell
viability [11], leaving no doubt for the physiological im-
portance of this protein outside mitochondria.

Dual subcellular localization has recently been suggest-
ed for another member of the Fe/S protein biogenesis sys-
tem, namely Dre2 (in humans also termed anamorsin or
CIAPIN1) [12]. The protein is an essential part of the cyto-
solic iron-sulfur protein assembly (CIA) machinery, and
together with its intimate partner, the diflavin oxidoreduc-
tase Tah18, forms an electron transfer chain that is crucial
for the assembly of cytosolic and nuclear Fe/S proteins [13,
14]. Dre2 itself is a Fe/S protein and was suggested to con-
tain two Fe/S clusters in its C-terminal domain which har-
bors two conserved cysteine-containing motifs [12]. Upon
discovery of Dre2, Zhang and colleagues noted that a small
amount of epitope-tagged Dre2 was associated with iso-
lated mitochondria. Since the protein was largely protease-
resistant, it was suggested to be located in the in termem-
brane space. However, verification of the presence of suc-
ch a co-

tion has been made by Peleh
man cells makes it unlikely that there are major dif-
ferences
between the high conservation of the CIA system in yeast an-
d hum-

not for modification by alkylating agents indicating that no
disulfide bridges were formed upon forced entry into the
intermembrane space, nor Fe/S clusters were coordinated.
How can this discrepancy to the earlier results [15] be ex-
plained? One notable difference may be the source of the
protein, yeast Dre2 versus human anamorsin. However,
the high conservation of the CIA system in yeast and hu-
man cells makes it unlikely that there are major differences
between these orthologues [14]. A more relevant sugges-
tion has been made by Peleh et al. [19]. Disulfide bridges
could in fact be introduced into insDre2 in vitro during the
isolation of mitochondria or upon treatment with the
chemical oxidant diamide. However, this oxidation step
was not observed, if the redox status of insDre2 was esti-

mated immediately during cell lysis and not after isolation
of mitochondria. From these observations, Peleh et al.
conclude that there is no indication for any redox modifica-
tion of the cysteine residues of Dre2 in vivo by Mia40 or for
the coordination of a Fe/S cluster in the mitochondrial in-
termembrane space [19]. Importantly, this data shows a
striking substrate specificity of the Mia40 oxidoreductase
for its native substrates typically containing twin CXXC or
twin CxX motifs [16]. In contrast, the twin CXXC motif pre-

sent in the C-terminal domain of Dre2 may not be a native

In an elegant biochemical effort the proteome of the
yeast mitochondrial intermembrane space has been identi-

ified [18]. Conspicuously, Dre2 was not contained in this list,
which simply may be due to the fact that minor constitu-
te may be missed in such systematic approaches.
Hence, it required a dedicated study to reinvestigate the
potential localization of Dre2 in this compartment. In lieu
of a true functional assay for Dre2 in the intermembrane
space, Peleh et al. decided to use two major strategies to
clarify the issue [19]. First, they carefully re-analyzed the
specific subcellular localization of Dre2. They indeed find
(overproduced) Dre2 located in the cytosol and associated
with mitochondria. From a number of technical approaches
including protease protection assays and sub-
mitochondrial fractionation the authors convincingly con-
clude that the protein is located outside rather than inside
the organelle. The mitochondrial surface-bound fraction of
Dre2 is protease-resistant as noted earlier, yet can be re-
leased by high salt.

In the second approach, Peleh et al. investigated
whether yeast Dre2 can use the Mia40 import pathway into
the intermembrane space. The first indication that this may
not be the case came from yeast Mia40 depletion experi-
ments. Efficient down-regulation of Mia40 did not af-
fect the amount of Dre2 associated with isolated mito-
chondria, a finding consistent with Dre2 sticking to the
outer face of mitochondria, yet inconsistent with a role of
Mia40 in Dre2 import into the intermembrane space.
Moreover, the C-terminal cysteine residues of Dre2 re-

ained reduced upon Mia40 depletion providing no indi-
cation that this oxidoreductase has any influence on the
amount and oxidation state of Dre2. Together, these re-
results rendered it unlikely that yeast Dre2 is a bona fide
constituent of the mitochondrial intermembrane space.

Peleh et al. then go on and take their study beyond a
mure protein localization study. They artificially (and quan-
titatively) direct Dre2 into the intermembrane space by
attaching it to the N-terminal targeting information of
Mia40 (residues 1-70). The fusion protein (imsDre2) follows
the classical presequence-dependent TOM-TIM pathway
[20, 21], and is hooked up to the inner membrane via the
Mia40 prepiece. When insDre2 was analyzed for its redox
status, all its cysteine residues were found to be accessible
for modification by alkylating agents indicating that no
disulfide bridges were formed upon forced entry into the
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twin CxX motifs [16]. In contrast, the twin CXXC motif pre-

sent in the C-terminal domain of Dre2 may not be a native
substrate of Mia40. While this finding provides interesting information on the substrate specificity of this oxidoreductase, the results also serve as a fine surrogate for ‘functional localization’ of Dre2, again arguing that in vivo the protein not normally enters and is processed in the mitochondrial intermembrane space.

The example of Dre2 provides a paradigm of how other cases of dual localization need to be viewed. The mere presence of a protein in a second compartment should not be regarded as a faithful hint that the protein is functional in this site, the more so if only miniscule amounts are present. Physiological relevance of the secondary localization can be assigned only after establishing the protein’s integration into a biologically relevant process within this compartment.

REFERENCES

CONFLICT OF INTEREST
The author declares no conflict of interest.

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