Mitochondrial Hsp70 and the troubles of nomenclature: leaving behind tradition to gain intuitiveness and clarity

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Accepted: 5 May 2016 / Published online: 22 May 2016
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In the age of OMICS life science, scientists are facing a huge amount of information on genes and gene products linked to an even larger number of acronyms that are familiar to members of inner circles while they are obscure for the outer circles and even more so for researchers crossing over from neighboring fields. Even if there was a one to one relationship between the descriptive names and the acronyms, it would not be easy to keep the necessary sets of proteins, genes, and acronyms in mind while navigating through talks and articles. Moreover, the historically created names and acronyms are not unique. There are many synonyms that allow different groups of researchers to discuss and publish without referring to other groups that work with the same molecule, just under a different name. It is difficult to change from a cherished, historically developed name and acronym that scientific networks have long used to a new one that has to be explained and linked to the earlier one. However, the changes have to be made if one wishes to let scientists outside the inner circles become interested in the subject and especially if one wishes to make the literature accessible for students and newcomers. For the acronym names of human genes, the Human Gene

Nomenclature Committee (<http://www.genenames.org/>) selects unique gene names and keeps watch on their use. Not all scientists fully adhere to this nomenclature, however, nor do they provide the synonymous acronyms to make it easier to see the connections and find articles in PubMed searches.

Despite these obstacles, we should try to implement some rules for the comprehensive use of names and acronyms to help researchers navigate this acronym jungle. Thus, the aim of this letter is to ask authors, peer reviewers, and journal editors to help implement the recommendations we give in Box 1 (Box 1). We will begin by using the mitochondrial heat shock 70 as an instructive example and will propose recommendations that could make life easier, promote understanding, and break down barriers to understanding between groups of researchers.

The Hsp70 family of molecular chaperones is an example of how historical designations and their uncontrolled proliferation have resulted in a confusing picture. Hsp70 family proteins are found in all subcellular locations and some of them are almost identical at the amino acid sequence level making it difficult to distinguish them at the protein level with antibodies and even by mass spectrometry. Many molecular chaperones received their names and acronyms in the historic wave of discovery of heat and stress induction, and they obtained the three letters Hsp for heat shock protein followed by a number referring to their molecular mass in kilodaltons as determined by SDS-PAGE. In most cases, this was before sequence information was available which could have helped to identify duplicates and organize them more systematically. Later, many homologs were identified, both in the same and in different organisms, and they received their analogous names based on what was known and available at the time. In spite of the suggestive “HSP” acronym, not all HSP’s are heat inducible, nor are they all chaperones. Another series of acronyms is

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based on the discovery of molecular chaperones that were upregulated upon glucose starvation of cell cultures. They received acronyms starting with GRP for glucose-regulated protein and a number for their apparent molecular mass. Some chaperones were independently discovered both as HSPs and GRPs and their identity was often first revealed when sequence information became available.

In humans, there are 14 genes encoding proteins belonging to the Hsp70 chaperone family. A systematic nomenclature for an unambiguous assignment of heat shock proteins including the Hsp70 family was recommended in a short communication in this journal (Kampinga et al. 2009). The systematic gene names for all human Hsp70 homologs start with *HSPA* followed by a digit and, in cases where the homologs have an almost identical amino acid sequence, by another letter. To link the proteins unambiguously to the “correct” gene, the gene names should also be used for the encoded proteins—using the same acronym but not in italics. The short communication by Kampinga et al. (Kampinga et al. 2009) has had a very positive effect, but further clarifications are needed.

The mitochondrial Hsp70 homolog has the highest number of pseudonyms and confusing names. By the late 1980s and early 1990s, evidence for a mammalian mitochondrial Hsp70 was reported at the protein level (Mizzen et al. 1989). These authors showed in rat fibroblasts and HELA cells that this Hsp70 homolog running at approximately 75 kDa in SDS-PAGE was induced by glucose starvation; hence, it received the designation GRP75 (glucose regulated protein with mass of 75 kDa). The same group showed subsequently that the GRP75 protein interacted with newly synthesized proteins in mitochondria (Mizzen et al. 1991). In 1993, a research group investigating proteins that are involved with and bind to peptides in connection with antigen presentation sequenced the complementary DNA (cDNA) encoding the human mitochondrial Hsp70, and, based on their targeting strategy, designated it PBP4 (peptide-binding protein 4) (Domanico et al. 1993). In the same year, a protein marker for cellular aging termed mortalin was described that became associated with the cytosolic fraction in serially passaged mouse embryonic fibroblast cells. Sequencing of its cDNA identified it as a Hsp70 family protein (Wadhwa et al. 1993), and further studies revealed that immortalized cells like the NIH3T3 fibroblasts expressed a perinuclear-localized genetic variant of mortalin carrying two amino acid changes in relation to the cytosolic form expressed in non-immortalized mouse embryonic fibroblasts. Cloning of the human gene and subcellular localization of the encoded protein established the relationship of these proteins with the mitochondrial Hsp70 protein (Bhattacharyya et al. 1995). Since then the different synonyms have been used alone or in combination making it difficult to collect the literature on this protein and its possible variants.

PubMed searches using the four different protein names for the *HSPA9* protein product give an instructive picture of the

problem (Fig. 1). GRP75 in the search field draws by far the most hits, followed by mortalin, HSPA9, and finally a few with PBP74. Furthermore, only ten of the articles are common to searches with all four names and only 49 of the 750 articles found with GRP75 can be detected also with mortalin as the search term. To obtain the full information, therefore, one has to search using *all* the acronyms.

Recommendations for a systematic nomenclature for heat shock genes and proteins (Kampinga et al. 2009) that committed the gene name *HSPA9* and the protein name HSPA9 have improved the situation somewhat. HSPA9 is now often used as a synonymous designation; however, earlier published articles have to be identified by doing searches for all the synonyms and pseudonyms. An example of the confusion arising from the different nomenclatures (evolutionary family (Hsp70/*HSPA*), regulatory relationship (heat shock/glucose starvation induced), size of the monomer in SDS-PAGE (66–78 kDa), and specific functional descriptor (peptide binding, mortalin)) is an article on the effects of overexpression of the mitochondrial Hsp70, i.e., HSPA9 (Xu et al. 2009). TRAP1/HSP75, names for the mitochondrial Hsp90 homolog, were here erroneously given as synonyms for HSPA9, probably because HSP75 has been confused with GRP75, the synonym for HSPA9.

When working with such protein families, it is relevant to check what is known on homologs in other organisms, especially the common model organisms, and to use model organisms for experimental studies. For the mitochondrial Hsp70, as for many other proteins, this opens another avenue of different acronyms and names that require translation and are in most cases not intuitive. In Table 1, the gene names and protein synonyms and names for classical model organisms are shown. For yeast, there exists a systematic nomenclature for heat shock 70 proteins that uses a yeast-specific nomenclature system: SS for stress seventy, a third letter for the subfamily (A, B, and C), and a digit to distinguish close paralogs (Craig et al. 1987). The yeast *Saccharomyces cerevisiae* mitochondrial Hsp70 SSC1 is the standard mitochondrial Hsp70 of this organism. It is essential for growth and has a broad functionality including protein import across the inner mitochondrial membrane, protein folding and refolding (Kominek et al. 2013). *S. cerevisiae* also contains two other mitochondrial Hsp70 family proteins: SSQ1, which has a more specialized function, namely iron-sulfur cluster assembly (Kominek et al. 2013) and SSC3, whose function is not well understood. In fruit flies (*Drosophila melanogaster*) and the model plant *Arabidopsis thaliana*, there are no intuitive nomenclatures, and one is left with the task of working out which Hsp70 family protein is which. *Caenorhabditis elegans* has a systematic nomenclature for open reading frames and the gene name *hsp-6* does not directly lead to the idea that this is the mitochondrial Hsp70 ortholog. Without knowing these relationships, it is difficult to find comprehensive useful information.

Fig. 1 Venn diagram of the distribution and overlap of hits for PubMed searches with HSPA9 and the aliases GRP75, mortalin, and PBP74. The PubMed ID's from hits were downloaded and compared using the software Venny (Oliveros 2007–2015). Numbers in brackets give the total number of hits for the respective queries

Finally, it remains difficult to detect the specificity of the respective Hsp70 in the forest of related forms with very similar primary sequences. Many studies offer incomplete records on the antibodies used and companies provide often ambiguous information on specificity, localization and other properties posing concerns. Hsp70s form a protein family and the sequence similarity between the different representatives (paralogs) within a given organism, as well as the sequence similarity between orthologs of different organisms, is high. This means that antibodies will often recognize more than one member of the family and they may or may not work in different tissues and distant species. Monoclonal antibodies have the advantage that they recognize one specific structure or sequence of amino acids. Polyclonal antibodies directed against peptides also restrict promiscuity and increase specificity. There are many good antibodies on the market and the sequence divergence between the mitochondrial and other Hsp70s in the c-terminal region usually allows for specific immunological detection. However, it is still necessary to record precisely which antibody was used and to elaborate on its specificity. Rigorous tests of specificity with regard to different paralogs and orthologs are almost impossible, but reference to the company and catalog number is helpful. Companies now also often provide lists of publications in which the respective antibody was used, which can assist in the judgment of specificity.

A specificity problem is also encountered when protein mass spectrometric methodology is used for identification and quantitation of Hsp70s. The high sequence conservation of Hsp70 family proteins from the same organism (Daugaard et al. 2007) causes peptides not to be unambiguously assigned

to one particular Hsp70 paralog. This affects quantitative data and one has to be aware of the algorithm used by the respective mass spectrometry software. Finally, the specificity of antibodies will become very important in the use of plasma HSPs as non-invasive biomarkers of diseases (Lu et al. 2015). HSPA9 has recently been found mutated in an inherited disease (Royer-Bertrand et al. 2015), and it has been implicated in the organization of an ER-mitochondria targeting complex (Paillusson et al. 2016; Szabadkai et al. 2006). There are likely interesting new aspects and more unexpected observations ahead, which require discipline in the use of names and acronyms to make the literature accessible. We therefore encourage authors to use and referees and editors to require the use of the recommendations in Box 1 not only for the mitochondrial Hsp70 HSPA9 but also for other genes and proteins.

Box 1. Recommendations:

- For human and mammalian proteins, the official name of the encoding gene should always be given in accordance with the HGNC recommended standard gene name.
- At first mention in the introduction, synonymous names and acronyms should be listed and appropriate references given.
- For model and other organisms, gene names, protein designations, and acronyms should be linked to the respective human homologs wherever possible.
- Antibodies should be described in detail in the materials and methods with specification of supplier, catalog number, and a descriptive phrase.
- Companies selling antibodies should precisely link the antigen used to a sequence database reference. Knowledge on specificity and cross reactivity should be described in detail.

Table 1 Gene and protein names for mitochondrial Hsp70 orthologs in different eukaryotic model organisms and *Escherichia coli*

Primary gene name	UNIPROT ID	Entry name	Protein names	Mass	Synonymous gene names	Organism
HSPA9	P38646	GRP75_HUMAN	Stress-70 protein, mitochondrial 75 kDa glucose-regulated protein (GRP-75) Heat shock 70 kDa protein 9 Mortalin (MOT) Peptide-binding protein 74 (PBP74) Stress-70 protein, mitochondrial (Uncharacterized protein) Stress-70 protein, mitochondrial 75 kDa glucose-regulated protein (GRP-75) Heat shock 70 kDa protein 9 Mortalin (MOT1 and MOT2) Peptide-binding protein 74 (PBP74) p66 Heat shock protein 9 (Hspa9 protein) Heat shock 70 kDa protein cognate 5	73,680	HSPA9 GRP75 HSPA9B mt-HSP70	<i>Homo sapiens</i> (human)
HSPA9	F1RGJ3	F1RGJ3_PIG		73,650	HSPA9	<i>Sus scrofa</i> (pig)
Hspa9	P38647	GRP75_MOUSE		73,461	Hspa9 Grp75 Hsp74 Hspa9a	<i>Mus musculus</i> (mouse)
hspa9	Q7ZYY3	Q7ZYY3_DANRE		73,894	hspa9	<i>Danio rerio</i> (zebrafish)
Hsc70-5	P29845	HSP7E_DROME		74,066	Hsc70-5 Hsc5	<i>Drosophila melanogaster</i> (fruit fly)
hsp-6	P11141	HSP7F_CAEEL	Heat shock 70 kDa protein F, mitochondrial	70,845	CG8542 hsp-6	<i>Caenorhabditis elegans</i>
SSC1	POCS91	HSP77_YEASX	Heat shock protein SSC1, mitochondrial Endonuclease Scl 75 kDa subunit (Endo.Scl 75 kDa subunit) mtHSP70	70,800	hsp70f C37H5.8 SSC1 ENS1	<i>Saccharomyces cerevisiae</i> (baker's yeast)
HSP70-9	Q8GUM2	HSP7I_ARATH	Heat shock 70 kDa protein 9, mitochondrial Heat shock protein 70-9 (AtHsp70-9) Mitochondrial heat shock protein 70-1 (mtHsc70-1)	73,075	HSP70-9 MTHSC70-1 At4g37910 F20D10.30	<i>Arabidopsis thaliana</i> (mouse-ear cress)
dnaK	P0A6Y8	DNAK_ECOLI	Chaperone protein DnaK (HSP70) Heat shock 70 kDa protein Heat shock protein 70	69,115	dnaK groP grpF seg b0014 JW0013	<i>Escherichia coli</i> (strain K12)

Acknowledgments Work on Hsps in RMT lab is supported by the Canadian Institutes of Health Research (CIHR) and the Natural Sciences and Engineering Research Council of Canada (NSERC). Work on mitochondrial chaperones and protein quality control in human diseases in PB lab is supported by the Ludwig og Sara Elsass Foundation.

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