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A Nuclear Function for Plakophilin-1 in the DNA Damage Response?

Mechthild Hatzfeld[1]

Plakophilins are proteins of the desmosomal plaque. Based on the observation that plakophilins localize not only to desmosomes but also to the cytoplasm and nucleus, additional functions in cell signaling have been proposed. In this issue, Sobolik-Delmaire *et al.* address the nuclear function of Plakophilin-1. The authors show that Plakophilin-1 interacts with ssDNA *in vitro* and may have a function in protecting cells from DNA damage.

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Desmosomes have long been regarded as structures that simply glue cells together and anchor the intermediate filament network. In line with this idea, inactivation of desmosomal components interferes with the structural integrity of tissues. Plakophilins were originally considered additional nonessential components of the desmosomal plaque, and the importance of plakophilins in desmosomal biogenesis and function has only recently emerged. Like γ-catenin/plakoglobin, plakophilins link desmosomal cadherins to the cytoskeletal linker protein desmoplakin. Moreover, one of the common characteristics of all plakophilins seems to be their ability to recruit desmoplakin to cell borders. Why epithelial cells usually express more than one Plakophilin- and how the plakophilins differ with respect to regulating adhesion are not entirely clear. Plakophilin-1 recruits large amounts of desmoplakin, desmoglein, and keratins to the membrane in agreement with its *in vivo* function in increasing desmosome size and strength in suprabasal cells of the skin (Hatzfeld *et al.*, 2000; Kowalczyk *et al.*, 1999).

Patients with null mutations in the gene that encodes Plakophilin-1 (ectodermal dysplasia/skin fragility syndrome) have skin-fragility defects and suffer from skin erosions and crusting as well as palmoplantar hyperkeratosis (McGrath *et al.*, 1997). At the molecular level, impaired recruitment of desmoplakin resulting in a reduced number of small and poorly formed desmosomes and cell–cell separation is observed. These pathological features point to an important role of Plakophilin-1 in stabilizing desmosome structure and function, predominantly in the spinous layer of the epidermis. However, other signs and symptoms of the genetic errors in Plakophilin-1 include abnormalities in ectodermal development with growth delay, hypotrichosis or alopecia, hypohidrosis, and nail dystrophy, which are not well understood.

Although so far no animal model for Plakophilin-1 deficiency has been described, Plakophilin-3 knockout mice have been generated. In these mice, desmosomes were altered in the basal layer of the epidermis and were essentially absent from the basal layer of the outer root sheath of hair follicles and from the matrix cells, leading to hair coat abnormalities. Moreover, Plakophilin-3 appeared to be involved in limiting the inflammatory response in the skin (Sklyarova *et al.*, 2008). The knockout of Plakophilin-2 revealed that this protein is essential for cardiac desmosome formation, which relies solely on Plakophilin-2, and heterozygous mutations in Plakophilin-2 are a common risk factor for arrhythmogenic right ventricular cardiomyopathy (Gerull *et al.*, 2004; Grossmann *et al.*, 2004). These observations indicate that plakophilins are essential for structural integrity and for modulating the composition and characteristics of desmosomes.

Plakophilins are found not only in cell contacts but also in the cytoplasm and the nucleus. We have a poor understanding of what regulates their localization and trafficking between the subcellular compartments and their nuclear function(s) in general. In this issue, Sobolik-Delmaire *et al.* address the question of what directs Plakophilin-1 into the nucleus. Using deletion mutants and chimeric proteins in which domains were nuclear localization signal–swapped between Plakophilin-1 and Plakophilin-3 (which reveals a much less pronounced nuclear localization compared with Plakophilin-1), the investigators identify a region in the Plakophilin-1 N-terminal domain that appears important for directing the protein to the nucleus. Because no classic nuclear localization signal can be identified, the mechanism by which Plakophilin-1 enters the nucleus remains unknown. The nuclear localization was apparent in proliferating HaCaT cells that lacked cell contact in the absence of Ca^{2+} but not in differentiated HaCaT cells, suggesting that the nuclear localization/function correlates with or even promotes cell growth.

Because Sobolik-Delmaire *et al.* (2010) were unable to identify nuclear proteins as Plakophilin-1 interaction partners, they hypothesized that DNA could be responsible for the observed Plakophilin-1 nuclear retention. Using *in vitro* affinity purification, they found an association of Plakophilin-1 with ssDNA cellulose but not dsDNA cellulose and also with dG-oligomers, supporting their hypothesis that Plakophilin-1 might associate with ssDNA in the nucleus. Although this is an intriguing observation, further tests and functional assays are required to validate this hypothesis. As a cautionary note, vimentin—an intermediate filament protein with a positively charged head domain—was also found to associate with ss- but not dsDNA *in vitro* (Traub *et al.*, 1992), but a functional relevance of this interaction

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could never be proven. Another question that arises in view of the recently described functions of Plakophilin-1 and 3 in translational control (Hofmann *et al.*, 2006; Wolf and Hatzfeld, 2010; Wolf *et al.*, 2010) is whether the preferential nucleic acid binding partner of Plakophilin-1 *in vivo* is indeed ssDNA or, instead, RNA. Unfortunately, RNA has not been tested as a putative binding partner. The interaction of Plakophilin-1 with components of the translational machinery was mediated by protein–protein interactions and was not dependent on the presence of RNA, as demonstrated by RNAse treatment in pull-down assays and by the association of Plakophilin-1 with translation initiation factors during affinity purification with the 7-methylguanosine (m^7GTP) cap structure (without an associated mRNA) as bait (Wolf *et al.*, 2010). This does not, however, exclude a putative interaction with RNA in the cytoplasm or the nucleus in living cells.

Based on the finding that Plakophilin-1 associated with ssDNA but not dsDNA, and considering that ssDNA occurs in the context of DNA repair, Sobolik-Delmaire *et al*. asked whether Plakophilin-1 might be involved in DNA damage response. Induction of DNA damage by etoposide (which forms a complex with DNA and topoisomerase II, thereby preventing religation of DNA strands) causes errors in DNA synthesis and promotes apoptosis of cancer cells. For this reason, etoposide is currently employed in cancer chemotherapy. Treatment of cells with etoposide induces a redistribution of Plakophilin-1 into subnuclear compartments identified as nucleoli by the investigators. Sobolik-Delmaire *et al*. speculate that this translocation of Plakophilin-1 to the nucleolus could suppress ribosome biogenesis under these conditions.

The nucleolus is the organelle of the cell nucleus where the transcription and processing machineries that are responsible for generating ribosome subunits are located. Nucleolar hypertrophy and increased ribosome biogenesis have been observed in all mammalian cells stimulated to proliferate, thereby meeting their need for high translation rates and increased protein synthesis. In fact, cell proliferation appears to be closely coordinated with nucleolar function. On the other hand, cells rapidly downregulate the synthesis of rRNA during the stress response. This is mediated by TIF-IA phosphorylation via JNK2 (c-*Jun* N-terminal kinase 2), which prevents the interaction of TIF-IA with RNA-Pol-I, inhibiting the transcription of rRNA (Boisvert *et al.*, 2007). Accordingly, TIF-IA was found to relocate from the nucleolus to the nucleoplasm in response to stress. A release of nucleolar proteins into the nucleoplasm upon exposure of cells to DNA damaging agents has been observed for other proteins, including poly(ADP-ribose) polymerase-1 (Rancourt and Satoh, 2009). Other proteins, however, appear to translocate into the nucleolus upon stress, as described for plant eIF4A-III, a putative component of the exon junction complex (Koroleva *et al.*, 2009). A possible explanation is that certain mRNAs remain bound to eIF4A-III under these conditions rather than being transported from the nucleus to the cytoplasm, thus preventing these mRNAs from being translated under certain stress conditions. Although this mechanism has not yet been validated in mammalian cells, it is tempting to speculate that a similar machinery could also work in the context of mammalian stress responses and that Plakophilin-1 could modulate this response via an interaction with eIF4A-III, a close relative of its interaction partner eIF4A-I (Wolf *et al.*, 2010).

The data presented in the paper by Sobolik-Delmaire *et al*. (2010) present an interesting and novel starting point for addressing Plakophilin-1 nuclear function. However, further experimentation is required to validate their findings and hypotheses and to address the functional relevance. The most important points relate to the specificity of the Plakophilin-1–ssDNA interaction and the nucleolar localization/translocation. Is the interaction specific for ssDNA or does Plakophilin-1 also interact with RNA? Is there any sequence specificity or sequence preference? Is the nuclear compartment to which Plakophilin-1 is translocated after cell stress indeed the nucleolus? Which types of stress can induce such a translocation? In addition, functional assays must address the role of Plakophilin-1 in ssDNA binding during DNA repair and its proposed role in suppressing ribosomal biogenesis after translocation into the nucleolus. Such studies will help to elucidate the role of the nuclear pool of Plakophilin-1 in genetic skin disease as well as resolve a putative contribution in tumorigenesis, as suggested by the findings that Plakophilin-1 (and Plakophilin-3) expression is elevated in some tumor samples (Wolf and Hatzfeld, 2010).

Clinical Implications

- Concepts of protein function have expanded with the new knowledge that a single protein may have two or more distinct roles within a cell.
- This expanded concept now includes the plakophilins, which were first identified as structural proteins of the desmosomal plaque.
- Based on the observation that plakophilins localize not only to desmosomes but also to the cytoplasm and nucleus, additional functions in cell signaling have been proposed.
- Sobolik-Delmaire *et al*. show that plakophilin-1 interacts with single-stranded DNA *in vitro* and suggest that it may even protect cells from DNA damage.

CONFLICT OF INTEREST
The author states no conflict of interest.

REFERENCES

Boisvert FM, van Koningsbruggen S, Navascues J *et al.* (2007) The multifunctional nucleolus. *Nat Rev Mol Cell Biol* 8:574–85

Gerull B, Heuser A, Wichter T *et al.* (2004) Mutations in the desmosomal protein plakophilin-2 are common in arrhythmogenic right ventricular cardiomyopathy. *Nat Genet* 36:1162–4

Grossmann KS, Grund C, Huelsken J *et al.* (2004) Requirement of Plakophilin-2 for heart morphogenesis and cardiac junction formation. *J Cell Biol* 167:149–60

Hatzfeld M, Haffner C, Schulze K *et al.* (2000) The function of Plakophilin-1 in desmosome assembly and actin filament organization. *J Cell Biol* 149:209–22

Hofmann I, Casella M, Schnolzer M *et al.* (2006) Identification of the junctional plaque protein Plakophilin-3 in cytoplasmic particles containing RNA-binding proteins and the recruitment of plakophilins 1 and 3 to stress granules. *Mol Biol Cell* 17:1388–98

Koroleva OA, Brown JW, Shaw PJ (2009) Localization of eIF4A-III in the nucleolus and splicing speckles is an indicator of plant stress. *Plant Signal Behav* 4:1148–51

Kowalczyk AP, Hatzfeld M, Bornslaeger EA *et al.* (1999) The head domain of plakophilin-1 binds to desmoplakin and enhances its recruitment to desmosomes. Implications for cutaneous disease. *J Biol Chem* 274:18145–8

McGrath JA, McMillan JR, Shemanko CS *et al.* (1997) Mutations in the Plakophilin-1 gene result in ectodermal dysplasia/skin fragility syndrome. *Nat Genet* 17:240–4

Rancourt A, Satoh MS (2009) Delocalization of nucleolar poly(ADP-ribose) polymerase-1 to the nucleoplasm and its novel link to cellular sensitivity to DNA damage. *DNA Repair (Amst)* 8:286–97

Sklyarova T, Bonne S, D'Hooge P *et al.* (2008) Plakophilin-3-deficient mice develop hair coat abnormalities and are prone to cutaneous inflammation. *J Invest Dermatol* 128:1375–85

Sobolik-Delmaire T, Reddy R, Pashaj A *et al.* (2010) Plakophilin-1 localizes to the nucleus and interacts with single-stranded DNA. *J Invest Dermatol* 130:2638–46

Traub P, Mothes E, Shoeman R *et al.* (1992) Characterization of the nucleic acid-binding activities of the isolated amino-terminal head domain of the intermediate filament protein vimentin reveals its close relationship to the DNA-binding regions of some prokaryotic single-stranded DNA-binding proteins. *J Mol Biol* 228:41–57

Wolf A, Hatzfeld M (2010) A role of plakophilins in the regulation of translation. *Cell Cycle* 9:1973–8

Wolf A, Krause-Gruszczynska M, Birkenmeier O *et al.* (2010) Plakophilin-1 stimulates translation by promoting eIF4A1 activity. *J Cell Biol* 188:463–71

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IL-17: Important for Host Defense, Autoimmunity, and Allergy?

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In this issue, Milovanovic and colleagues present evidence that IL-17a enhances IgE production, although the precise mechanism remains unclear. Their initial finding was that higher numbers of IL-17a-producing CD4$^+$ T cells were observed after polyclonal stimulation in a largely airway allergic population. These data add to the evidence that atopic disorders such as asthma and, possibly, atopic dermatitis (AD) may have distinct immunologic phenotypes. The hope is that by characterizing the immunologic basis of these common diseases we will be able to understand the heterogeneity observed in natural history, response to treatments, susceptibility to infections, genetic risk factors, and associations with other atopic disorders.

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IL-17 history and function

IL-17-producing T cells were initially described in autoimmune models (Aggarwal *et al.*, 2003; Cua *et al.*, 2003; Murphy *et al.*, 2003) and led to the characterization of a new T helper (Th) subset that extended our repertoire beyond Th1, Th2, and Th9. Th17 cells are thought to be associated with the development of autoimmune diseases, response to extracellular pathogens, and diseases characterized by chronic neutrophilic inflammation. IL-17a, one of the most studied Th17 cytokines, was first recognized for its innate immune actions (Fossiez *et al.*, 1996), consistent with its rapid production in response to microbial infections. For example, IL-17 has been shown to play a crucial role in host defense against *Staphylococcus aureus* skin infections (Ishigame *et al.*, 2009). This is consistent with the observation that T cells from subjects with hyper-IgE syndrome, which is characterized by recurrent and often severe pulmonary infections, eczema, staphylococcal abscesses, and mucocutaneous candidiasis, do not produce IL-17 (Milner *et al.*, 2008). Studies in recombinase activating gene (RAG)-deficient mice, which lack B and T cells, demonstrated that IL-17a could still be produced in response to IL-23, suggesting that there were non-T-cell sources for this cytokine (Uhlig *et al.*, 2006). This work was followed by multiple publications that demonstrated several cell types found within skin and mucosal surfaces produce IL-17a as part of an innate immune response (Cua and Tato, 2010). Therefore IL-17a can be considered part of both an innate and an adaptive immune system.

Immunologic classifications of allergic disorders

Allergic disorders affect up to 30% of the populations in developed countries and are caused by aberrant immune responses to allergens and other environmental stimuli. Current thinking has led to the recognition of at least two variants referred to as "atopic" (or extrinsic) and "nonatopic" (or intrinsic), which is probably best studied in asthma. Atopic asthmatics have more Th2-type airway inflammation characterized by IL-4-, IL-5-, and IL-13-secreting cells that result in tissue eosinophilia and increased serum IgE levels, whereas nonatopic asthmatics are characterized by increased IL-8-producing cells and tissue neutrophilia without elevations of IgE (Amin *et al.*, 2000). It has been suggested that IL-17a expression observed in some asthmatics is responsible for the tissue expression of CXCL8 and neutrophilia that is characteristic of the nonatopic variant. All individuals with atopic dermatitis (AD) are characterized by a lack of

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