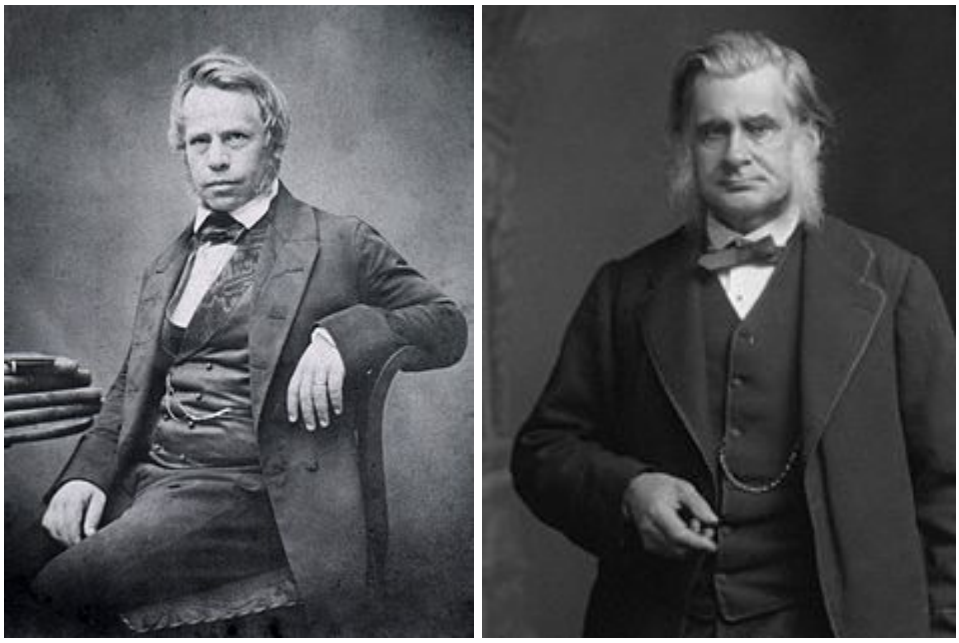
Hair-raising Mysteries of the Inner Root Sheath: Unearthing Treasures in Bald Spots

- The inner root sheath (IRS) is pigeon holed as a structural component of the hair follicle.
- Our review of the IRS suggests a broader signaling function.
- IRS signaling could play a role in folliculogenesis, anagen prolongation, cell differentiation, immunomodulatory function, and keratinization.



Jacob Henle discovered the IRS in 1840 while Thomas Huxley discovered Huxley's layer in 1845

Introduction:

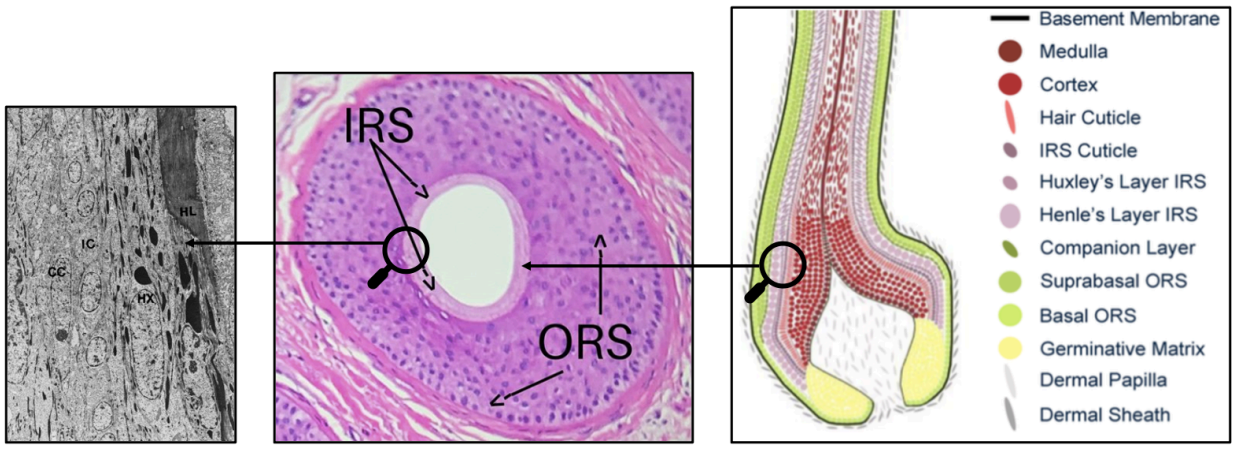
The IRS of the human hair follicle was first discovered in 1840 when the German anatomist Jacob Henle generally described the structure (Henle, 1840). Just five years later in 1845, Thomas Huxley detailed an additional layer within the IRS now referred to as Huxley's layer (Joshi, 2011). Despite the relative breakneck pace of research in the 19th century, there has been shockingly little progress understanding the IRS since. While the mainstream accepts the structural and protective function of the IRS, there is a notable lack of research on its alternative signaling functionalities.

Anatomy of the Inner Root Sheath:

The IRS is composed of three main layers namely, the cuticle, Huxley's layer, and Henle's layer. All three layers of the IRS originate around Auber's line (Auber, 1952), which is a precise line across the widest portion of the dermal papilla. The cuticle is the innermost layer of the IRS and consists of a single layer of anucleated, squamous, overlapping cells which form a shingled pattern with their free edges pointing down (Montagna & Ellis, 1958). The shingled cells of the IRS cuticle interlock with the hair shaft cuticle cells whose free edges point upward. Huxley's layer is the next innermost layer of the IRS and it is approximately two to four cells thick, largely

composed of the specialized protein Trichohyalin and Flügelzellen cells (also known as winged cells). Trichohyalin plays a critical role in linking keratin filaments together during keratinization while Flügelzellen interlock Henle's layer with the companion layer of the outer root sheath (ORS). Clemmensen & Hansted, 1991 speculated that Flügelzellen may also be involved in cell differentiation or nutrient transport to the companion layer/ORS. Counter to mainstream understanding, the companion layer may actually be a fourth layer of the IRS or its own layer independent of the ORS as it remains fully intact on plucked hair follicles whereas the ORS is completely lost (Langbein et al., 2002b).

IRS morphogenesis begins during early to mid anagen as cells derived from the hair matrix differentiate and begin taking shape (Montagna & Ellis, 1958). Notably, all three layers of the IRS become hyalinized, which is the process of becoming acellular and translucent, at different points in their progression (Patil et al., 2021). Henle's layer begins hyalinization immediately from the matrix and is fully hyalinized by the upper bulb section of the hair follicle. Hyalinization does not begin in Huxley's layer until the top of the hair bulb and the cells are not fully hyalinized until the midpoint of the hair follicle. Cuticle cells only begin hyalinization at the midpoint of the hair follicle where all layers of the IRS fuse together, forming an undifferentiated mass of hyalinized cells. The IRS abruptly ends around the pilosebaceous canal, potentially due to selective activity from keratinase or some other enzyme (Stankovic et al.1929). Curiously, the IRS disappears in its entirety during mid to late catagen (Paus et al. 2016) leaving an open question: where does it go?



Hair Follicle Anatomy Zoomed in on the IRS

Accepted Functions of the Inner Root Sheath:

Mainstream hair follicle research accepts that the IRS plays a crucial role protecting, supporting, and guiding the hair follicle upward as it ascends towards the epidermis (Martel et al., 2022). Robins & Breathnach, 1970 observed that during its earlier stages of development, the advancing tip of the hair cone is entirely formed by trichohyalin, Huxley, and Henle layers of the inner root sheath. Montagna & Ellis, 1958 described the IRS and hair shaft growing upward at approximately the same rate, supporting the idea that the harder IRS is able to penetrate the skin and pull the softer hair shaft towards the epidermis during folliculogenesis.

Researchers also broadly accept the IRS's critical role in keratinization of the hair follicle. During keratinization, trichohyalin granules populate the cellular region and recruit various keratins and proteins (Clemmensen et al., 1991). As the process continues, keratinizing cells lose their nuclei and other organelles, which are replaced by intermediate filaments, primarily composed of keratins and other proteins. Keratinized cells become flattened and tough, allowing them to serve their protective function against external elements.

Non-traditional Inner Root Sheath Functionality:

Besides the commonly accepted functionality of the IRS, it appears that mainstream researchers may have overlooked complex signaling functionality. As we'll specify in the next section (and in even further detail in our table) the IRS is loaded with biomarkers that suggest signaling functionality involved in early-stage cell differentiation, immunomodulatory activity, anti-oxidative activity, and even organogenesis. Even less broadly accepted, Paus et al., 2020 suggest that Flügelzellen of the IRS could carry noxious compounds from the ORS into the hair shaft, serving an excretory function.

Evidence to support cross-talk between the IRS and other compartments of the hair follicle is supported by findings like Enshell-Seijffers et al., 2018 who found Wnt5a highly expressed in the dermal papilla of murine hair follicles until IRS formation began at which point Wnt5a expression became nearly undetectable. Fuchs et al., 2014 also noted in murine hair follicles that after IRS formation, Shh signaling from matrix cells stops being transmitted to the dermal papilla. Both cases demonstrate that signaling from the IRS has a direct impact on gene expression in other compartments of the hair follicle, further dispelling the notion that the IRS only provides structural support.

Inner Root Sheath Protein Expression:

As detailed in our table of IRS protein expression, researchers discovered a flurry of different keratins expressed within the IRS during the early 2000s. The primarily understood function of these keratins is to confer strength to the IRS. Amid the flurry of new keratin discoveries, Langbein et al., 2001 observed that keratins form distinct patterns amongst themselves, potentially influenced by their status as type I (acidic) or type II (basic) or via some form of external signaling. Interestingly, Foxn1 and LEF-1 are expressed within the IRS and were identified for their role in keratinization. Thus, it appears likely that the IRS plays a critical role in initiating the keratinization process. However, we are left to wonder - is it possible that these keratins have signaling capacity outside of their structural functionality? Research is required.

The IRS also expresses several BMP proteins and Wnt ligands during folliculogenesis, known for their roles in organogenesis, anagen initiation/prolongation, and metabolic regulation (Hogan et al., 2000, Millar et al., 2001). While their expression appears to subside around mid-anagen, the evidence suggests that IRS signaling could play a meaningful role in folliculogenesis before transitioning to its structural role as the hair follicle enters steady state. Supportive of this type of

IRS functionality, Paus et al. 2010 found Thyrotropin-Releasing Hormone Receptor (TRH-R) primarily expressed in the IRS of human hair follicles while TRH was predominantly expressed in the ORS, suggesting IRS metabolic involvement and crosstalk between the two compartments.

In juxtaposition to the IRS's pro-growth signaling, we also found evidence of the IRS negatively regulating cell differentiation. Fuchs et al., 2018 found HES1 and GATA3, known negative regulators of cell differentiation, expressed within the IRS of mouse hair follicles. Fuchs et al.,2014 found pSMAD1/5 expression during mid-anagen in the IRS of mouse follicles, which is also known to be a negative regulator of cell differentiation. The ability of IRS signaling to shift from pro-growth to anti-growth demonstrates a marked versatility not often associated with this structural compartment.

Other non-traditional signaling functionality suggested by our IRS protein analysis includes stress-sensing, heat-sensing, and immunomodulatory activity. Tobin et al. 2020 found a variety of proteins associated with stress sensing and negative regulation of cell differentiation in the IRS of an in vitro human hair follicle. Song et al., 2021 noted that TRPV3 is strongly expressed in the IRS of mouse hair follicles. Davis et al., 2002 discussed the heat-sensing functionality of TRV3 and detailed its role in triggering an immune response. Sakata et al., 2006 found an association between elevated TRPV3 expression and mast cell degranulation in hairless mutant rodent strains.

Diseases and Morphologies Associated with the Inner Root Sheath:

The IRS is implicated in several diseases and morphologies which hint at its wide array of functionality. Related to its roles of structural support and anchoring, IRS dysfunction is implicated in both Uncombable Hair Syndrome and Loose Anagen Syndrome. Uncombable Hair Syndrome is characterized by the curly/frizzy hair phenotype and is associated with TCHH, PADI3, and TGM3 mutations - all of which are expressed by the IRS (Westgate et al., 2017). Without dysfunction, our referenced proteins would have driven the keratinization process. Likewise, Loose Anagen Syndrome can be characterized by fine and sparse hair follicles that, despite being in the anagen growth phase, can be frictionlessly and painlessly pulled out of the head. Interestingly, Maxfield & Cook, 2023 remark that the IRS is nonexistent in Loose Anagen Syndrome despite citing Mirmirani et al., 2010 which characterizes the IRS as disfigured rather than nonexistent. Nonetheless, both findings implicate the IRS in a role of providing support and shaping the hair shaft while also anchoring it to the scalp.

Centrifugal Cicatricial Alopecia (CCCA) and Olmsted syndrome are also associated with IRS dysfunction and provide more support for our hypothesis that the IRS has signaling functionality. CCCA is a form of scarring alopecia, particularly common in African women, which causes bald patches on the vertex and/or crown of the scalp (Tan et al., 2019). The fact that IRS dysfunction triggers scarring in CCCA, implies the necessity of the protein expression associated with anti-inflammatory effects and cell differentiation referenced earlier. Olmsted syndrome is an extremely rare disease, as only 73 cases had been reported worldwide as of Duchatelet &

Hovnanian, 2015. Olmsted syndrome is characterized by the combination of palmoplantar keratoderma, periorificial plaques, hair loss, and is driven by mutations of the TRPV3 gene (Song et al, 2021). The TRPV3 mutation triggers premature differentiation of follicular keratinocytes while inhibiting cell differentiation in the IRS, driven by decreased expression of several proteins including Foxn1, Msx2, Dlx3, and Gata3. IRS involvement in both CCCA and Olmsted syndrome support the hypothesis that IRS signaling plays a crucial role in regulating cell differentiation and the inflammatory response.

Perspectives & Conclusions:

Exploring the existing literature on the inner root sheath (IRS) in human and animal hair follicles has revealed known structural functionalities while also hinting at an unexplored signaling role. With additional research, we may find that IRS signaling plays a crucial role in folliculogenesis, later-stage cell differentiation, and immune response. Further studying the IRS, particularly its gene expression changes throughout the hair follicle life cycle, may provide fascinating insights which could prove clinically and translationally relevant for patients with a variety of scalp disorders. However, a critical research gap over the last two decades has prevented us from gaining a deeper understanding of the IRS. Given the strides in technology which now allow us to take high resolution pictures and evaluate protein expression in various tissues, the IRS may serve as a new frontier in our increasing understanding of the human hair follicle.