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Newsletter for April 2024

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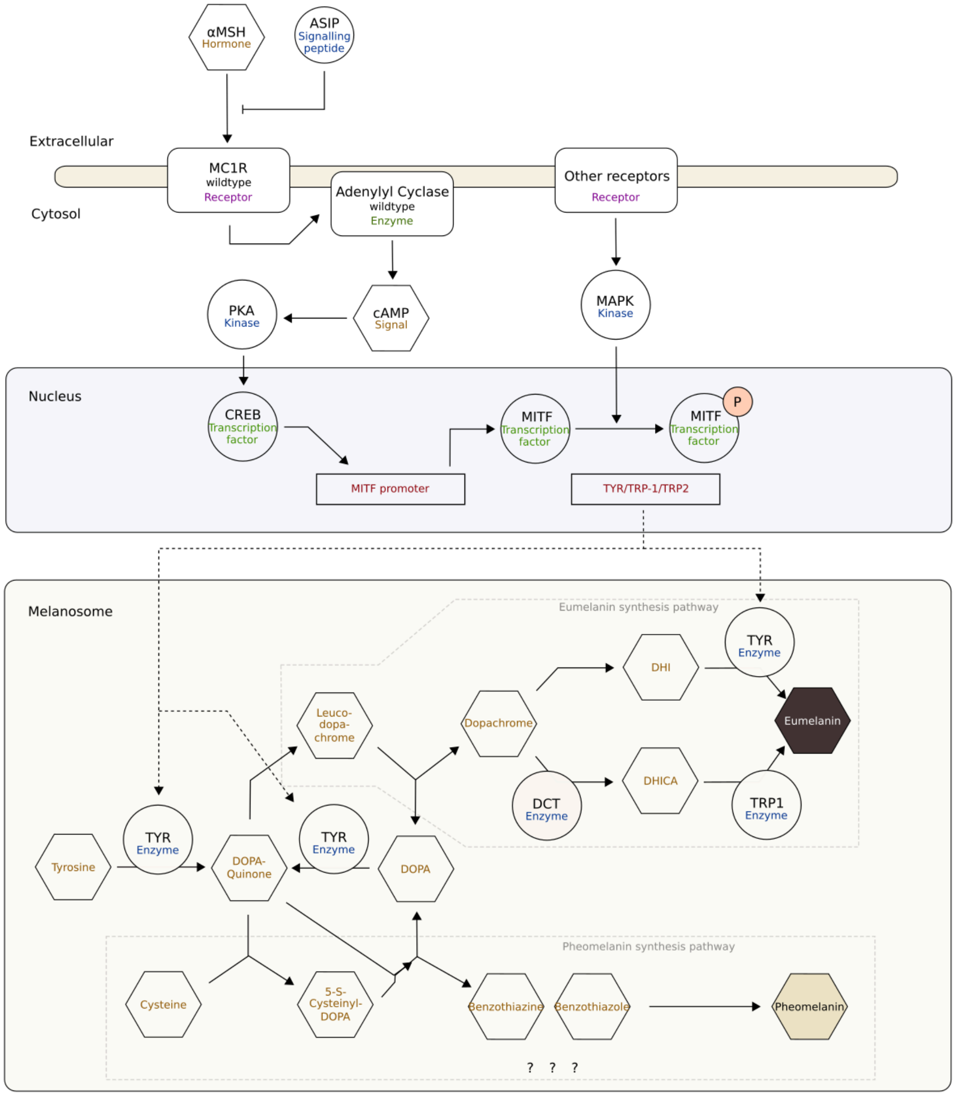
Integument Review Progress Update

By Lathreas & Zennith | April 28, 2024

The skin is one of the most visible areas that will be affected by our modifications - namely, for the creation of fur, scales, and feathers. The integumentary structures we create should feel and look natural, be sufficiently robust to stress, and be capable of replacement or regeneration cycles without needing continuous medical upkeep. Our Integument Review Project is taking a detailed look at how to create those features. More specifically, in order to successfully engineer the appropriate tissues, we need to understand the basic biology of the cells and molecules involved, and then follow up on that basic foundation with hypotheses and specific engineering plans for each needed integumentary feature.



Our work is still progressing, and we are starting to get closer and closer to releasing Chapters 2 and 3 of the Integument Review, which are the Properties of Feathers and Properties of Scales. At the same time, we are working on Chapters 5 and 7, which are about the development of human integument and development of feathers, respectively.

Developmental tuning of pigments

Of course, knowing what to change or add to human skin is only part of the battle. Importantly, we must also find ways of how to accomplish that. For the most comprehensive results, we must make ourselves deeply familiar with the genetic circuits that control the formation of all of the structures we see. Chapters 5 to 8 are focused on the developmental biology and gene regulatory

Figure 1 (previous page): Work in progress diagram of melanin production activation by MC1R, to be verified and expanded. By Lathreas.

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aspects of integument. In Chapter 5, we are investigating what knobs we can turn to change pigments, and in later chapters we will tackle more colorful, non-mammalian pigments as well.

Even so, human pigments alone already cover a large range: mammals only express eumelanin and pheomelanin pigments, and those are innately present in humans. It is a ‘simple’ matter of turning on the production of these pigments such that we get the right color of fur, and that way we can reconstruct whatever luxurious colors are found in any other mammal. Importantly, the concentration of the pigments must be just right, because the overall color is a function of how much pigment is present. Only a little pigment, and it’s light yellow to orange. A lot, and it’s deep brown to black. You can read our Chapter 1 (which you can download from https://freedomofform.org/research/project-integument-review/) with more details on the basics.

From a developmental standpoint, we must therefore know how the production of these pigments are turned on or off, and how to control such a system to stabilize the concentration. Interestingly, the genetic network that governs this is widely researched. After all, a big function of the pigmentation system is to protect DNA against UV radiation, and as such, it has gained interest in the wider scientific community. We, however, are primarily interested in how pigments are formed using developmental cues, i.e. how each cell ‘knows’ when to produce pigment in certain spots of the body, but not others.

In humans, for example, the shorter *vellus* hairs are not pigmented, whereas *terminal* hairs generally are pigmented: in the same human, the formation of pigments is turned on in some cells and it is turned off in others. This shows clearly that there is a knob we can turn; some signal we can give or change to activate pigment formation.

One important part of the network controlling pigment formation is depicted (Figure 1). It involves the *MC1R* protein: a receptor protein that listens to various signals and enables the production of melanin when activated. It does so by activating the transcription factor *MITF*, which in turn activates the expression of *Tyrosinase* (TYR), an enzyme that is important in many steps of the melanin synthesis pathway.

There are two ways to get to the desired concentration of melanin: either there is a short burst of melanin production, before production is reduced or turned off, or there is some stable balance between production and degradation that the system approaches over time.

This exposes many interesting questions, which we aim to investigate next: can we predict the final melanin concentration of a cell based on its control circuitry? Which of the proteins inside the regulatory network provide a good candidate for robust control? And finally, what is the effect of cell proliferation on the overall melanin content inside a tissue, and hence the overall color?

The more we know about the network at hand, the better we will be able to create mathematical models to predict the system, or devise experiments that can alter the network’s behavior. This will ultimately let us control the color of skin, and more importantly, fur.

Scale coloration

Another aspect we are investigating in detail is the coloration of scales. The way coloration is formed here is quite fascinating! Although we often think of lizards, and many other reptiles, as being primarily green, it turns out that they do not, in fact, have any green pigment, nor is the green color produced by mixing blue and yellow pigments like in paint.

The main pigment in green lizard skin is just yellow pigment. So then, why do they look green?

The key here is *iridophores*, which are specialized colorful cells inside a reptile's skin. They do not contain any pigments, but they do contain arrays of crystals, such as guanine stacks. Because these crystals are arranged in a regular microscopic pattern, they begin to interfere with the light waves, forming what is known as a *Bragg mirror*. Such mirrors reflect light due to constructive and destructive interference depending on the spacing between the crystals, and reflect only some wavelengths back in what some of you may recognize as *iridescence*. This indeed is what gives iridophores their name: they are iridescent chromatophores, and in lizards, the spectrum that is reflected is shifted towards blue. In combination with the yellow pigments, this creates the green colors we all know and love.

Importantly, the light that is reflected has certain properties that simple pigments won't have. For example, the reflected light has a slight metallic sheen the 'more blue' it tends to be, giving reptile scales their unique non-plasticy look. Chameleons, in fact, use the special properties of iridophores to dynamically change the color of their skin. By changing the salt concentration in iridophores, the crystals will move further apart or closer together, resulting in different wavelengths being reflected back. This is visible as a dramatic change in skin color, which can be achieved in very short timescales. Fish skin in turn is often specularly reflective due to extensive use of iridophores as opposed to simple pigments.

All of these effects rely on iridophores being the base layer of scale coloration. Light that falls in will reflect off of this 'mirror' in the back, and gets further colored by pigments that lie on top. Indeed, if the pigments would be behind this mirror, they would get obscured, and as such, if we want to reconstruct compelling scales, it is important that these chromatophores are correctly positioned in the skin.

This leaves us with an interesting engineering challenge, and there are several key questions we must tackle: first, we must learn how to introduce the aforementioned Bragg mirrors into human skin, and secondly, we must ensure that they get introduced in the right layer of the skin. Perhaps the likeliest and most realistic route to getting this to work would be to use an approach similar to setting a tattoo, which uses a tool to insert novel compounds deep into the skin — we must precisely control the depth if we do so, and we must use a type of 'tattoo ink' that is reflective in similar ways as iridophores, perhaps by using the very same guanine crystals that lizards and fish use as the 'ink'. More futuristic options may include the genetic engineering of cell lines to produce natural Bragg mirrors like in reptiles and fish; attractive, but incredibly challenging. Even so, it's not unthinkable. All options are still on the table, and we are carefully investigating what the effects would be of any strategy we pursue. Stay tuned!

Feather growth
We are also working to understand the fundamental biological processes for how the integument develops and grows naturally. The more we understand about these systems, the better we can engineer them.

This is expected to be especially true for feathers, which have to go through very complex steps of growth during embryogenesis. Feathers are non-living structures once complete, but during their growth process, there's a lot of moving parts that influence the ultimate type, shape, and coloration of feathers that are produced.

Does this matter, though? Before going down this rabbit hole of understanding the biological process, we wondered whether it could make sense to simply implant artificial feathers for avian patients, or those desiring a feathered form. However, feathers (whether artificial or real) would accumulate damage over time, and need to be replaced periodically — perhaps annually across the whole body. We don't think that annual implants of feathers across the whole body is likely to be a practical choice, so we are strongly leaning towards specifying that feathers need to be produced from living feather follicles, and naturally replaced by molting.

With this in mind, we indeed needed to understand how feathers grow. So, we rolled up our sleeves and dove into a lot of reading.

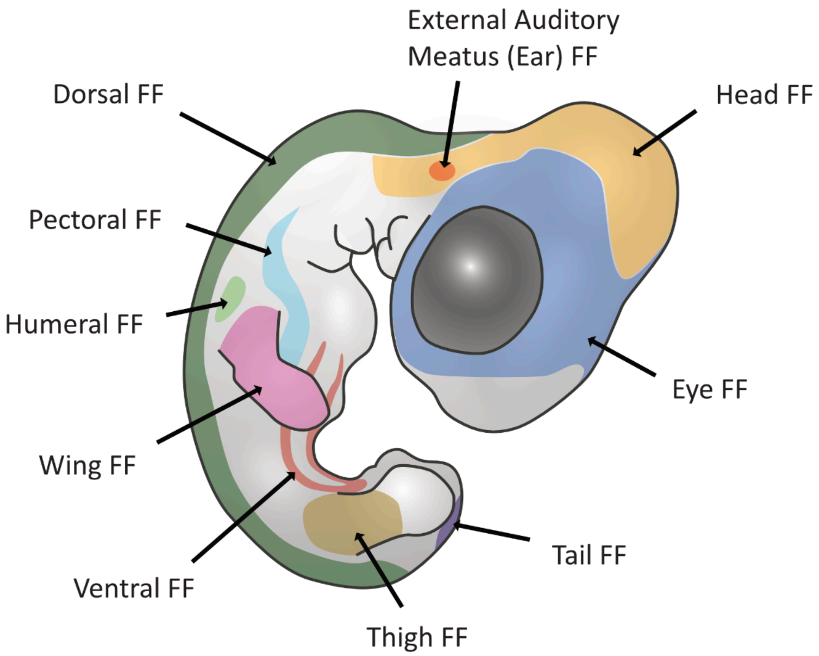


Figure 2: Feather fields (FF) in a developing chick embryo. By Zennith.

Each region of the body has distinct types of feathers, and these large-scale regional differences are some of the earliest decisions to be made in embryonic development through something called macropatterning (Figure 2). Then, micropatterning follows, which determines the exact placement of each individual feather that will eventually form — though, at this time, they only look like bumps of cells!

Those bumps become feather buds, which soon form *feather follicles* as well as *feather filaments*. The feather follicle is basically just a mounting point for the growing feather. But the feather filament... well, this is where things really get interesting. A feather filament is a tube of living cells sticking out of the skin, whose cells are proliferating from the root of the follicle.

The feather filament is extruded upwards over a long period of time, and during the extrusion process, the cells inside are communicating with each other, forming more complex structures within the filament.

The filament looks like a simple tentacle until the very end of growth. The appearance is misleading, because a lot is happening inside. Cells towards the anterior side of the filament may form the structural core of the mature feather, the rachis (also known as the 'stem' of the feather). Still other cells, reaching around towards the other side of the filament, undergo a process called *ramogenesis* and start forming the feather's barbs, which are the protrusions branching off of the rachis. Whereas, the inside of the filament is living pulp, which is where blood supply for all the actively growing cells come from. The outside of the filament is called the sheath, which keeps growing as well, protecting the delicate structures inside until they are mature and strong enough.

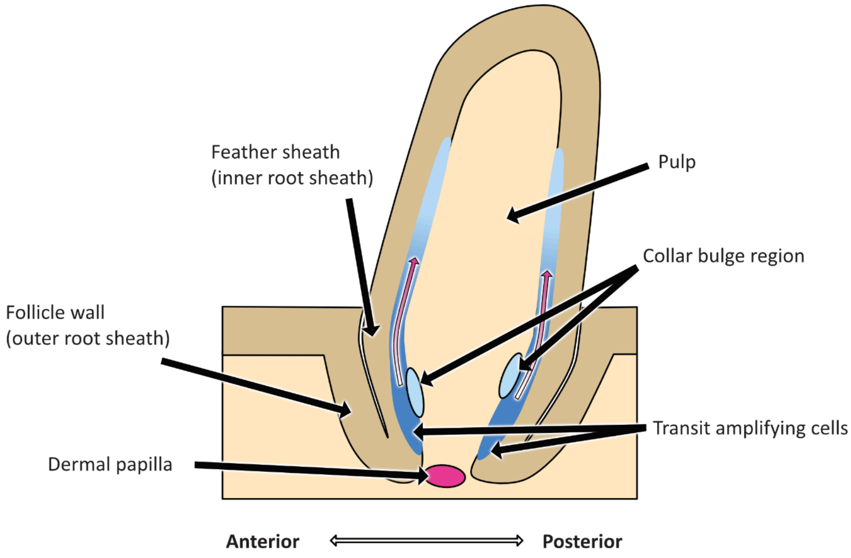
As the rachis reaches maturity and ramogenesis reaches completion, cells that are between each barb are no longer needed and undergo programmed cell death. Cells in the rachis and barbs undergo terminal differentiation, filling up with keratin and becoming non-living, strong structures. Once the feather is mature, the bird prunes the sheath off, and the feather inside unfurls into its final form.

Note that while macropatterning, micropatterning, and follicle formation are not repeated in adult feather regeneration, the feather growth process itself is repeated.

So, how can we use this knowledge? Well, we have no intention of exactly recapitulating the embryonic development of feather formation. But, knowing how the tissues determine the type, shape, and orientation of feathers is essential. We now know, for example, that the type of feather (e.g. whether or not it's a flight feather) is information stored within the feather follicle. And we know that, in nature, the anterior side of the follicle will be associated with the rachis, so when we engineer follicles, we will need to be careful about how they are oriented.

Two main stem cell populations are involved in these things (Figure 3). The dermal papilla is a relatively simpler one, responsible for generating the feather's internal pulp. Whereas, the collar bulge is more interesting. The collar bulge is a ring-shaped stem cell population going all around the feather follicle's base. A tilt in the collar bulge — i.e., the ring not laying 'flat' in the follicle — is associated with flight or contour feathers, in fact! Whereas, collar bulges that lay 'flat' are associated with downy feathers. The collar bulge contributes transit amplifying cells which rapidly proliferate and are responsible for generating the feather's ultimate structure. Transit amplifying cells create the rachis and barbs mentioned above.

Figure 3: Growing feather follicle and filament, with some cell populations marked. By Zennith.



So, from all of this, we know that we at least need follicles that:

- store information about the type of feather the follicle is responsible for;
- are oriented properly; and
- have stem cell populations that are capable of running the subsequent processes involved in feather filament formation — making the rachis, barbs, sheath, and pulp.

With those processes in mind, it's now a lot more meaningful to think about the specific genes and signaling pathways in mind. And reviewing those specifics is next on our agenda for this chapter!

After those specifics are nailed down, we will be in the position where we can start engineering them. Again, we will not need to recapitulate embryonic development for feathers.

We don't need to reproduce macropatterning, micropatterning, or bud formation. We can "cheat" our way to essentially complete feather follicles, with the needed stem cell populations, using bioprinting. By having a good understanding of the underlying biology, and by carefully isolating individual problems, generating complete feathers from engineered follicles will be tractable. Not necessarily easy, but absolutely tractable.

Again, we're aiming to get Chapters 2 and 3 out soon, and we plan on following up in several more weeks with Chapters 5 and 7. Things are on track and we can't wait to share them with you!

Thank you as always to the folks who support our work, whether financially, being in our community, or being a source of new ideas or insight. And that includes everyone reading this newsletter article - it's very energizing knowing that people find it interesting and useful. We always appreciate the support and can't do this without you!

For more detail about our Integument Review Project, you can always go to: https://freedomofform.org/research/project-integument-review/

Volunteer Spotlight: Keiro Ituralde

By SvarOS | March 31, 2024

An interview with foundation board member and Chief Information Officer (CIO) Keiro Ituralde

About:
His pseudonym is Keiro Ituralde, one that he plans on making official at some point. Currently located in the state of Alabama in the US at present. His career in IT started fairly early on when he gained access to his family's Windows 95-based computer, this experience carrying on into his career. The rest, as he says, is history.

Beyond that, his interests include reading, especially enamored with sci-fi, fantasy, high fantasy, and other fantastical tales like the Dresden Files, the Cosmere, Green Rider series, and so many more. He's also an avid biker, electronic tinkerer, technician, and more.

SvarOS (Interviewer): So for those that may not interact with you, who are you?

Keiro: I'm Keiro, a systems administrator by trade and personally, a deaf advocate. I manage information technology for the foundation.

Interviewer: What does doing your job look like to people who may be unfamiliar with what you do?

Keiro: I'm a technical support operator for my night job, it's mainly fixing servers and websites for customers but for the Foundation, I take feedback on what's needed now and in the future and plan out how that looks like. For example, we eventually have plans for our own labs or access to one and that requires lots of careful thought and research.

Interviewer: Who do you work with that helps you maintain that?

Keiro: That would be my employer, the work is mostly involved in maintaining services such as the website, the applications that support the website and generally covering where I'm not available.

Interviewer: What do you find the most challenging aspect of your work? Is it something technical or is it something else?

Keiro: Coming up with ways of making the best of what we have and understanding what our more scientist-oriented staff need. A lot of the terms in use are over my head at times. For me, though, it's making sure the data is accessible to those that need it, as we also make extensive use of a vendor's services. We do have plans for our own data services but that's a very complex subject at this time.

Interviewer: I imagine there have been some fires that you've put out for the organization. Is there anything where you or your team rose to the occasion to solve the issue?

Keiro: I think the most fire we had was a day long attack on services that were mitigated by the protections I put in place for that purpose. The attack was a DDoS [Distributed Denial of Service] hitting the website and the initial configuration I had in place did anticipate some form of the attack, but didn't fully account for it. Fortunately, it was not my first rodeo. We have tools for that, and common procedures and switching to an alternative allowed the server to react with much larger loads.

Interviewer: What does the foundation's success look like to you?

Keiro: I think that's the advocacy we're working on now where we're providing support to those who wish to realize the dream that the foundation has. But... By and large, for the future, I think the successes we have will be in making our research impactful in ways we have yet to see. The easy answer would be for us to become real furries. The hard answer, I think lies in convincing those in the broader society that this is a legitimate thing.

Interviewer: How long do you think we'll need to achieve success?

Keiro: I think a few more years. We're already making impressive progress since incorporating in. I think it was 2018, but our research is already showing results. I suspect this will be more visible within the next 5 years. More interest, specifically. We've produced at least one research paper but if my understanding of the science field is correct, usually producing more such papers that are considered credible it's a sign that we've done something right.

Interviewer: What do you find the most satisfying about your work with the foundation?

Keiro: Getting to hear the thanks from people who genuinely love what we do. It's all in the magic of the furry world. Better yet, seeing one's face light up in pure joy is incomparable, in my opinion.