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Flash forward genetics: new twists in transcription across evolutionary boundaries

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"Flash forward genetics" refers to a genetic approach based on the functional interaction of a given factor with unknown partner(s) converging on shared targets across evolutionary boundaries. A study by Li *et al* (2021), published in this issue of *EMBO Reports*, illustrates the innovative potential of the approach. The authors applied it to identify interacting factors for FOXN1, a mammalian transcription factor with a highly specialized function in hair follicle morphogenesis and thymus. The authors express FOXN1 in the *Drosophila* eye to perform an unbiased genetic screen in a totally heterologous system. In a remarkable *tour de force*, the authors identify and characterize a factor so far known for its ubiquitous function in transcription elongation, AFF4. Li *et al* show that AFF4 plays also a specific role in hair follicle and thymus development in the mouse overlapping with that of FOXN1.

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See also: **J Li *et al*** (March 2021)

There is no need to emphasize the great power of *Drosophila* genetics, which made major inroads in understanding the complex mechanisms behind organ morphogenesis and homeostasis. Dissection of genetic networks in this organism has led to the discovery and functional elucidation of key signaling pathways with global as well as specific implications for human health and disease. A baffling variation of biological systems has emerged since the origin of life and the surge of eukaryotic cells and multicellular organisms. Yet, multiple divergent branches of the evolutionary tree are based on the same or very similar organizational principles, as a result of shared origins and/or convergent evolution.

Hair-, feathers-, and scales-bearing organisms have a common origin (Dhouailly, 2009). Their amniote ancestors, having stably moved to land in the late Carboniferous (~300 million years ago), diverged in two lineages, the synapsids, from which modern mammals are derived, and the sauropsids, precursors of reptiles and birds. While the same or similar signaling pathways are utilized for morphogenesis of these hard keratinized structures, the types of keratin by which they are composed are significantly different. Cysteine-rich alpha keratins provide the backbone of hair ("hair keratins"), while totally distinct keratins, ß-keratins, are present in scales and feather (Dhouailly, 2009). A master regulator of hair keratin expression is FOXN1/WHN, which was identified in 1994 as one of the mutations responsible for the mouse and rat nude phenotypes (Nehls *et al*, 1994).

FOXN1 is a member of the large family of Forkhead box (FOX) transcription factors, found in all organisms from yeast to man, sharing a similar winged-helix DNA-binding domain, but having otherwise divergent structures and functions. As Li *et al* point out, FOXN1 has no homologue of functional significance in *Drosophila*. The most closely related fly FOX family member, Jumu (Dom, Dwhn), shows only 14% similarity in overall amino acid sequence, with different DNA-binding specificity and biological functions (Hofmann *et al*, 2010). In fact, the origin of the *Foxn1* gene can be traced to vertebrates, with the *Foxn4* gene coding for a highly related paralogue with very distinct roles during development (Bajoghli *et al*, 2009). Interestingly, the differences between *Foxn1* and *Foxn4* may be more related to the different tissues in which they are expressed than intrinsic biochemical functions, as *Foxn4* expression under the *Foxn1* promoter can rescue the nude mouse phenotype to a large extent (Swann *et al*, 2014).

In the hair follicle, FOXN1 is primarily required for hair keratinization and terminal differentiation of the epidermis. The complexity of the hair follicle has been revealed by a recent single cell RNA sequencing study that led to the identification of several cell populations distinguishable by differential gene expression (Joost *et al*, 2020). In agreement with FOXN1 function in hair keratinization, this transcription factor is expressed in a highly specific fashion in the most terminally differentiated cells of the hair cortex and cuticle, which form the external layers of the hair shaft. Its expression is also observed in other three of the 24 clusters of the hair follicle, as

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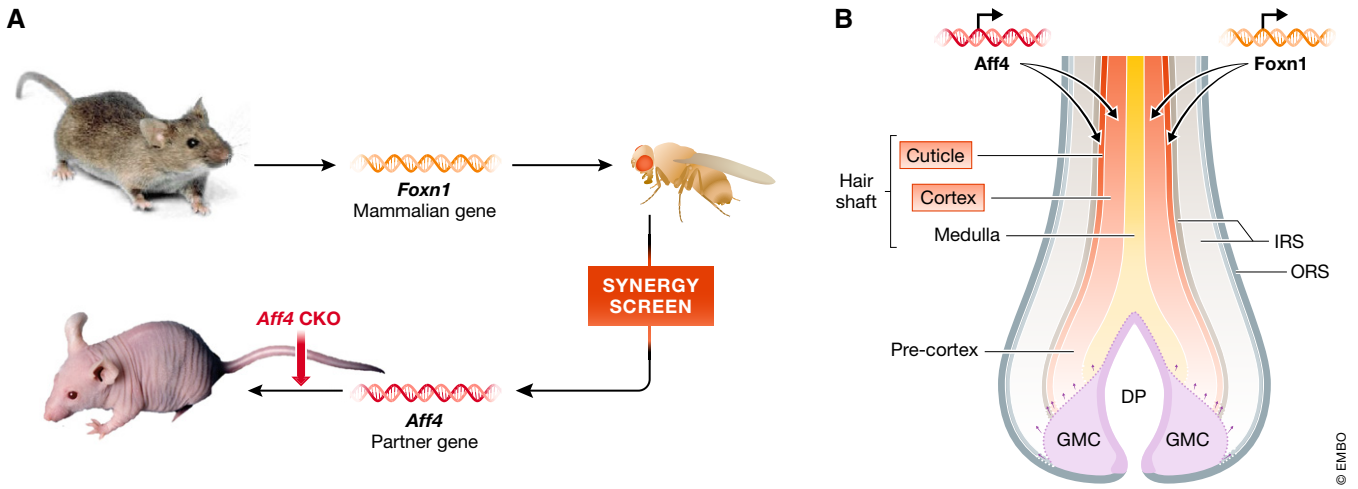

Figure 1. Flash forward genetics: from mice to flies and back.
(A) Schematic illustration of the combined genetic approach employed by Li *et al*, with the identification of *Aff4*, a component of the super-elongation complex, as a novel tissue-specific determinant of gene expression converging with the *Foxn1* transcription factor. (B) *Aff4* and *Foxn1* are both expressed in the cuticle and cortex of the hair shaft. IRS, inner root sheath, ORS, outer root sheath, GMC, germinal matrix compartment; DP, dermal papilla.

well as in the granular layer of the epidermis, consistent with its function in the epidermal differentiation program.

In addition, FOXN1 plays an essential role in differentiation of the thymic epithelium and in T-cell specification. Recent single cell RNA sequencing analyses have revealed a more complex and heterogeneous scenario than previously anticipated, demonstrating that thymic epithelial cells are composed of dozens of molecularly distinct cell populations clustered in five major molecular sub-types (Bornstein *et al*, 2018). *Foxn1* is primarily expressed in the cortex and in one of the four major medulla cell clusters.

By a sophisticated series of genetic and biochemical/approaches Li *et al* establish AFF4, a nearly ubiquitous component of the transcription super-elongation complex, as a novel factor converging with FOXN1 tissue-specific functions in both hair follicle and thymus (Fig 1). By molecular characterization of constitutive and conditional double knock-out models, the authors show that FOXN1 and AFF4 play an unexpected convergent role in both tissues. In skin, they regulate an overlapping set of target genes, including hair keratins and the chemokine CXCL12, a well-characterized determinant of T-cell specification under FOXN1 control in thymus (Zuklys *et al*, 2016), suggesting a functional conservation of this duo of proteins in both tissues. It should be noted, however, that AFF4 positively regulates only a subset of FOXN1 regulated genes in the skin, indicating an additional level of specificity.

The stunning similarity between the phenotypes of nude mice and of epidermis-specific conditional *Aff4* knock-out mice, combined with the highly significant overlap of transcriptomic alterations found in skin, leave no doubt about the specificity and significance of FOXN1 and AFF4's functional interaction. Previous work has highlighted the well-established role of AFF4 as a scaffold protein of the transcription super-elongation complex (SEC) (Takahashi *et al*, 2011), and no other functions have been reported. AFF4 physically interacts with several SEC components via its intrinsically disordered N-terminal region. While no direct binding with FOXN1 was observed in the present study, AFF4 was previously shown to interact with components of the mediator complex such as MED26 (Takahashi *et al*, 2011). Therefore, an attractive possibility is that its interactions with FOXN1 may occur through the mediator complex which is known to bridge transcription factors bound at enhancers with the proximal promoter. Another intriguing question for further studies is whether, in parallel with its functional convergence with FOXN1 in skin and thymus, AFF4 may also participate in the tissue-specific functions of the FOXN1-paralogue FOXN4. Other genetic co-determinants of FOXN1/4 function in mammalian cells and tissues may result from further analysis of other interactors predicted from the *Drosophila* screening assay. Finally, in keeping with its evolutionary origin, FOXN1 is expressed across vertebrate phyla, raising the intriguing question of its possible convergence with the ubiquitous AFF4 in a large variety of organisms.

The work from Li *et al* highlights the significance of *Drosophila* and other basic genetic systems for the analysis of mammalian genes. These convergent studies help to shed light not only on conserved functions but also on mammalian-specific inventions and could also help to understand the establishment of boundaries across evolution.

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References

Bajoghli B, Aghaallaei N, Hess I, Rode I, Netuschil N, Tay BH, Venkatesh B, Yu JK, Kaltenbach SL, Holland ND *et al* (2009) *Cell* 138: 186 – 197

Bornstein C, Nevo S, Giladi A, Kadouri N, Pouzolles M, Gerbe F, David E, Machado A, Chuprin A, Toth B *et al* (2018) *Nature* 559: 622 – 626

Dhouailly D (2009) *J Anat* 214: 587 – 606

Hofmann A, Brunner M, Schwendemann A, Strodicke M, Karberg S, Klebes A, Saumweber H, Korge G (2010) *Chromosome Res* 18: 307 – 324

Joost S, Annusver K, Jacob T, Sun X, Dalessandri T, Sivan U, Sequeira I, Sandberg R, Kasper M (2020) *Cell Stem Cell* 26: 441 – 457 e447

Li J, Lee YK, Fu W, Whalen AM, Estable MC, Raftery LA, White K, Weiner L, Brissette JL (2021) *EMBO Rep* 22: e49804

Nehls M, Pfeifer D, Schorpp M, Hedrich H, Boehm T (1994) *Nature* 372: 103 – 107

Swann JB, Weyn A, Nagakubo D, Bleul CC, Toyoda A, Happe C, Netuschil N, Hess I, Haas-Assenbaum A, Taniguchi Y *et al* (2014) *Cell Rep* 8: 1184 – 1197

Takahashi H, Parmely TJ, Sato S, Tomomori-Sato C, Banks CA, Kong SE, Szutorisz H, Swanson SK, Martin-Brown S, Washburn MP *et al* (2011) *Cell* 146: 92 – 104

Zuklys S, Handel A, Zhanybekova S, Govani F, Keller M, Maio S, Mayer CE, Teh HY, Hafen K, Gallone G *et al* (2016) *Nat Immunol* 17: 1206 – 1215