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Telomeres: A New Means to an End

Gene duplication provides an important evolutionary mechanism for functional diversification. A new study in *Drosophila* indicates that gene duplication has allowed telomere protection to be partitioned between the soma and the specialized chromatin environment of sperm.

Justin Blumenstiel

Differential gamete size defines sperm and eggs and this distinction defines the male and female sexes.
The evolution of multicellularity in sexually reproducing species often results in the evolution of gamete dimorphism [1]. This is because diverse modes of selection such as competition between gametes and the challenges of fertilization favor abundant tiny sperm and provisioning oocytes. To achieve small size, a tremendous degree of chromatin compaction must occur within sperm and this is accomplished through the removal of chromatin-bound histones and their replacement with basic nuclear proteins such as protamines [2]. Despite the nature of this highly specialized chromatin landscape within sperm, essential chromosome function must be maintained. In the case of telomeres, the ends of chromosomes must be protected from degradation and from fusion by the DNA repair machinery. How is telomere protection achieved across chromatin landscapes that differ so greatly between sperm and soma? In a recent issue of *Current Biology*, Dubruille *et al.* [3] provide a fascinating example of how gene duplication can provide a resolution to this problem in *Drosophila*.

Telomeres in most eukaryotes are composed of simple repetitive sequences maintained by telomerase. By contrast, *Drosophila* telomeres consist of retroelement arrays maintained by retroelement reverse transcriptase [4]. Within the soma, these unusual chromosome ends are protected by an assemblage of capping proteins designated HP1, HOAP and HipHop [5,6]. Using cytological and genetic approaches, Dubruille *et al.* [3] have now demonstrated that instead of HipHop, *Drosophila* rely on K81 for telomere protection within sperm. *K81* was originally described in *Drosophila* as a member of a rare class of paternal effect mutations [7,8]. Homozygous males produce motile sperm capable of fertilization, but embryos undergo early arrest caused by failure of the male pronucleus to participate in early nuclear divisions. Remarkably, phylogenetic analysis indicates that *K81* arose from a *hiphop* retrotransposition event in the ancestor of the *D. melanogaster* subgroup (Figure 1). Subsequently, *K81* and *hiphop* diverged rapidly from each other and have evolved reciprocal functions in telomere protection. HipHop is adapted to maintain telomeres within the soma whereas K81 maintains telomeres within the unusual chromatin landscape of sperm. Through a series of experiments, the authors demonstrate that K81 is necessary for maintaining HP1 and HOAP at paternal telomeres in fertilizing sperm. In wild-type flies, K81 is maintained on paternal chromosomes until just after the first zygotic mitosis after which it is soon

replaced on newly replicated paternal chromosomes by maternal HipHop. In the absence of K81, HP1 and HOAP are lost from telomeres during spermatogenesis. Paternal chromosomes fail to segregate properly in the first zygotic mitosis and the authors propose this arises from telomeric fusions of unprotected paternal chromosomes.

What forces selected for this specialization following gene duplication? The authors suggest that the chromatin landscape of sperm poses a unique challenge at telomeres and that gene duplication allowed *K81* to follow an evolutionary path of divergence uncoupled from its *hiphop* progenitor. Two models describe how gene duplicates can be maintained within species. One model proposes that gene duplicates are retained by neofunctionalization [9]. In particular, one copy retains the ancestral function while the other copy evolves a newly selected function. A second model hypothesizes that the two gene copies undergo subfunctionalization through reciprocal degeneration [10]. In this model, the original gene function is retained, but only due to the persistence of two complementary copies. For the most part, the persistence of *K81* and *hiphop* as gene duplicates appears to be a canonical case of subfunctionalization since an ancestral gene capable of protecting telomeres in all tissues seems to have partitioned telomere protection across different tissue domains. This is consistent with regulatory subfunctionalization leading to tissue-specific gene expression followed by complementary degradation of protein function. Notably, K81 and HipHop proteins are unable to protect telomeres when their expression is driven outside of their resident tissue domains. However, it is difficult to explain the evolution of K81 and HipHop by subfunctionalization alone. If K81 exhibits specialized ability to assemble telomere-capping proteins on chromatin containing protamines in sperm, neofunctionalization (defined biochemically) has clearly evolved. Furthermore, from a strict regulatory perspective, novel function is suggested since the retrotransposition event giving rise to *K81* placed the gene copy into a new regulatory domain. Overall, the K81/Hiphop system exemplifies how it may be difficult to attribute the persistence of duplicate genes either to subfunctionalization or neofunctionalization, and that such characterization depends on how gene function is defined. Finally, to completely understand the evolution of these duplicated genes, ancestral function must be determined. Both K81 and HipHop protect telomeres, so it is reasonable to assume the ancestral function was also to protect telomeres. Since *K81* arose through retrotransposition into a new regulatory domain, it is also reasonable to conclude that its specialized function in spermatogenesis is derived. However, it is formally possible that this specialized function is in fact ancestral and that HipHop general function is derived. Determining HipHop function in outgroup species that diverged from the *D. melanoagster* lineage prior to gene duplication will provide insight into evolutionary trajectories following the origin of K81.

Whether subfunctionalization, neofunctionalization, or a combination of the two most fully explains the evolution of *K81*, it is notable that sperm function seems especially influenced by gene duplication events. For example, in a global analysis of rates of gene duplication by retrotransposition, many were found to be involved in male fertility [11,12]. Whole-sperm proteomics has also revealed gene duplications yielding new sperm proteins [13]. Dubruille *et al.* [3] suggest that the unique chromatin environment of sperm explains the persistence and specialization of the duplicate *K81* gene. However, multiple evolutionary forces such as sexual selection and genetic conflict act on sperm, and these may additionally influence duplicate gene evolution, including *K81*. Interestingly, the retroelement telomeres themselves may impose other modes of selection on K81. The machinery of RNA silencing by piRNA has been shown to be important for maintaining telomeres in the male germline [14], and the piRNA machinery itself is evolving under strong positive selection, likely due to the evolutionary arms race it finds itself in with transposable elements within the germline [15,16]. Specialization of the telomere maintenance machinery on the part of K81 may be influenced by the extent to which it, alongside the retroelement arrays, is a hostage to this

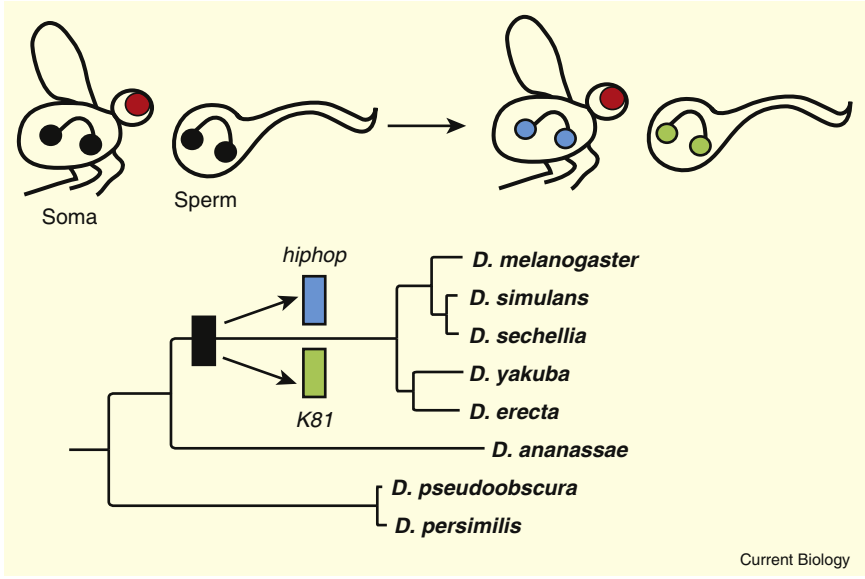

Figure 1. Evolution of telomere protection in *Drosophila* sperm.

A retrotransposition of the *hiphop* gene gave rise to a new gene, designated *K81*, in the ancestor of the *D. melanogaster* subgroup. Subsequent to this duplication, functional and regulatory divergence has resulted in the partitioning of telomere protection. HipHop protects telomeres in somatic tissues, whereas K81 functions within the unusual chromatin environment of sperm.

evolutionary conflict. In light of this, it is significant that other components of the telomere-capping complex evolved rapidly within *Drosophila* [5].

The study by Dubruille *et al.* [3] is a critical demonstration of how evolutionary and functional studies can mutually inform one another. By characterizing the function of K81, they have provided a remarkable example of how gene duplication can lead to functional specialization of telomere-capping proteins in the male germline. With the great advances in technology over recent years, it seems likely that joint evolutionary and functional approaches such as this will continue to yield exceptional insight.

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Developmental Biology: Extending the Limb and Body with Vectors and Scalars

Outgrowth of the embryonic limb in vertebrates is driven by a proximodistal gradient of cell movement, with WNT and FGF activities controlling direction and velocity, respectively. A similar gradient, though without a directional bias, drives caudal body axis extension.

Mark Lewandoski and Susan Mackem

The outgrowth of the posterior axis of vertebrate embryos has often been speculatively compared to outgrowth of the appendages. Indeed, there is considerable overlap in terms of the genetic regulation of both processes. But how the genetic networks instruct the cellular behaviours that drive axis and limb bud extension has remained unclear. Three papers [1–3], one of them in a recent issue of *Current Biology*, now address this issue, pointing to conserved mechanisms of cell movement. Although two of these papers make some use of established techniques such as labelling specific lineages with vital dyes, the core data of all three papers are generated by tracking cell behaviour with sophisticated time-lapse video microscopy as development proceeds. This approach is possible due to the toolkit of genetically encoded fluorescent probes now available to label cells within a tissue or a subset of structures within individual cells [4], enabling the determination of cellular orientation, division plane and velocity [5].

In all vertebrates, the limb bud first arises as a local swelling in the lateral plate mesoderm. Soon after this initial bud formation, a thick columnar structure arises, along its distal margin, called the 'apical ectodermal ridge' (AER). Fibroblast growth factors (FGFs), in particular FGF8, from the AER signal to the underlying mesenchyme and are essential for limb bud extension and patterning [6]. During outgrowth, the limb bud first forms as a hemispherical swelling that then becomes a flat and markedly elongated structure. This process has been traditionally perceived as driven by anisotropic growth that is spatially graded proliferation at a high rate in the distal bud mesenchyme, driven by mitogenic activity of AER-derived FGF. On the other hand, AER-specific gene inactivation studies in the mouse have revealed that although loss of FGF signalling results in a much smaller bud, it causes no clear proliferative or cell survival defect. On the basis of this observation, the Martin lab speculated that a lack of AER-FGF signalling might impair cell movement in limb bud initiation [7].

Boehm *et al.* [5] have recently addressed the validity of the growth-driven morphogenesis model using