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Note

A Key Transcription Cofactor on the Nascent Sex Chromosomes of European Tree Frogs (*Hyla arborea*)

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ABSTRACT

We show that MED15, a key component of the transcription complex Mediator, lies within the nonrecombining segment of nascent sex chromosomes in the male-heterogametic *Hyla arborea*. Both X and Y alleles are expressed during embryonic development and differ by three frame-preserving indels (eight amino acids in total) within their glutamine-rich central part. These changes have the potential to affect the conformation of the Mediator complex and to activate genes in a sex-specific way and might thus represent the first steps toward the acquisition of a male-specific function. Alternatively, they might result from an ancestral neutral polymorphism, with different alleles picked by chance on the X and Y chromosomes when MED15 was trapped in the nonrecombining segment.

A widely accepted scheme for the evolution of Y chromosomes assumes that the initial acquisition of sex-determining genes is followed by the suppression of recombination over a so-called differential segment, which then progressively expands from the sex-determining region to the chromosome extremities. The nonrecombining segment covers for instance only 1% of the young (<10 MY) medaka fish Y chromosome (Kondo *et al.* 2004; Zhang 2004), but encompasses the whole Y chromosome in humans (except for tiny pseudo-autosomal regions), after >200 MY of mammalian sex-chromosome evolution. Expansion may proceed in phases, creating evolutionary strata of different X–Y divergence (Charlesworth *et al.* 2005).

The suppression of recombination preserves associations between alleles with advantageous epistatic fitness effects, which may occur for two reasons. First, sex determination may depend on several genes (Haldane 1922; Nei 1969; Charlesworth 1991, 2002), in which case recombination would produce intersex individuals with lowered fecundity. Second, sex differentiation may involve genes with sex-antagonistic effects (*e.g.*, advantageous for the heterogametic sex, but deleterious for the homogametic one), so that recombination would generate segregation loads (Rice 1987, 1996). Several poeciliid fishes, for instance, have color genes located on the nonrecombining segment, in close linkage with sex-determining genes, because the bright colors favored in males by sexual selection would be detrimental in females (review in Lindholm and Breden 2002).

The suppression of recombination, however, has other consequences for the proto Y chromosomes, including enhanced drift, background selection, and selective sweeps. In addition, genes with no sex-specific effects that happen to be trapped in the nonrecombining segment are kept in a state of permanent heterozygosity in the heterogametic sex. Deleterious mutations with sufficiently small effects will thus tend to accumulate in such genes, leading to their progressive decay (Charlesworth and Charlesworth 2000; Steinemann and Steinemann 2005). Several genes trapped in the recent Y-specific region of the medaka fish have already become nonfunctional (Nanda *et al.* 2002), and ancient Y chromosomes have lost most of their functional genes, except for a few with sex-specific effects. The human Y chromosome, for instance, retains only ~100 functional genes, many of which are expressed only in testes.

Fully fledged sex chromosomes are well documented, but, except for a few recently investigated cases (*e.g.*, McAllister and Charlesworth 1999; Filatov *et al.* 2000; Bachtrog 2004; Charlesworth 2004; Kondo *et al.* 2004; Liu *et al.* 2004; Peichel *et al.* 2004), we know little about young sex chromosomes, which have the potential to deliver important insights into the evolutionary fate of genes recently trapped in nonrecombining regions. Does the Y copy rapidly decay under the accumulation of deleterious mutations or does it

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develop new, male-specific functions in relation to sex determination or sex differentiation?

All amphibians tested so far have genetic sex determination, but heteromorphic sex chromosomes are rare (<4% of 1500 species studied so far; Schmid *et al.* 1991; Eggert 2004). Nothing is known about amphibian master sex-determination genes and very little about sex-chromosome evolution (Schartl 2004). The European tree frog *Hyla arborea* has homomorphic sex chromosomes (Anderson 1991), but male heterogamety was recently revealed by the sex-specific patterns of a microsatellite-like marker (Berset-Brändli *et al.* 2006, 2007). At locus *Ha5-22*, all females investigated were homozygous for allele 235 and all males were heterozygous for alleles 235/241, pointing to a location within the nonrecombining region of nascent sex chromosomes, with allele 235 fixed on the proto X and 241 on the proto Y.

The successful amplification of *Ha5-22* in several hylid species (Berset-Brändli *et al.* 2006) as well as the nature of its tandem repeat (a trinucleotide CAG repeat) suggested a location within the coding region of a conserved gene. To address this point, we analyzed the mRNA expression of *Ha5-22* in *H. arborea* embryos at different developmental stages by cDNA reverse transcription (Methods in supplemental material). After an initial period of purely maternal expression (only the X copy was detected within 24 hr of clutch deposition), both alleles were found to be expressed later (stage 23 and 30, Gosner 1960) in heterozygous larvae (*i.e.*, males). To identify the gene containing *Ha5-22*, we sequenced the cDNA of both X and Y alleles from three male and three female larvae from a single clutch (stage 23), by walking from the known *Ha5-22* sequence toward the 5′ and 3′ directions. The sequences obtained were identified by BLAST analysis as homologs of MED15 (also known as ARC105 or PC2QAP or TG1; Bourbon *et al.* 2004), a key component of the Mediator coactivator complex (Conaway *et al.* 2005). The HaMED15 protein (supplemental Figure S1) is 81, 66, 59, and 51% identical to MED15 of *Xenopus laevis*, *Gallus gallus*, *Homo sapiens*, and *Danio rerio*, respectively, each harboring highly conserved N- and C-terminal ends and a central glutamine-rich (polyQ) domain.

The X allele (2376 bp, 792 aa) and Y allele (2382 bp, 794 aa) differed only by three frame-preserving indels, all within the polyQ domain (Figure 1): one 9-bp insert (QQQ) in the X allele at position 248, one 6-bp insert (QQ) in the Y allele at position 305, and one 9-bp deletion (QAQ) from the X allele at position 506. Insertions *vs.* deletion events were inferred from the repeat motives and comparison with the *X. laevis* DNA sequence (supplemental Figure S2). The 6-bp indel in position 305 corresponds to the *Ha5-22* polymorphism previously described (alleles 235 and 241; Berset-Brändli *et al.* 2006). Consistency of the two 9-bp indels in positions 248 and 506 was confirmed by screening

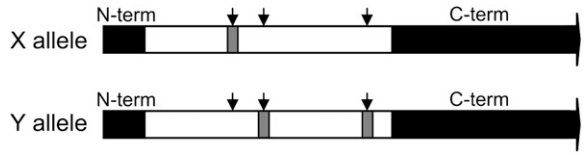

Figure 1.—Schematic of the X and Y *HaMED15* alleles with their conserved N-terminal (KIX-domain) and C-terminal regions (solid boxes). Arrows and shaded boxes indicate the three sex-specific frame-preserving indels within the central polyQ region. Detailed methods and sequences appear in supplemental material.

genomic DNA from 18 adults (10 females and 8 males) with specific primers. Besides these indels, no single nucleotide difference between X and Y alleles was observed, suggesting a very recent divergence. No polymorphism was found among either the X or Y copies, but all individuals sequenced derived from the same clutch, so that the power to detect polymorphism was null on Y and very low on X.

Coactivators play key roles as interfaces between the gene-specific regulatory proteins (activators) and the basal transcription machinery (TFII and RNA polymerase II). Mediator is a multisubunit complex, responding to different activators by adopting specific conformations (Taatjes *et al.* 2002), which enables it to perform series of specialized roles (*i.e.*, trigger-specific genes). In its active form, Mediator consists of three modules: head (eight subunits), middle (eight subunits, binding to the transcription machinery), and tail (five subunits, binding to the activator). MED15, a subunit of the tail module, binds to different activators through its conserved N-terminal 3-helix bundle (KIX domain; Yang *et al.* 2006).

Targeted activators include Smad2/4, which plays a key role in transforming growth factor β (TGFβ), and Activin/Nodal signal transduction, a pathway that exerts profound effects on cell migration and differentiation. MED15 expression was shown to stimulate axis duplication and mesoderm differentiation in *X. laevis* (Kato *et al.* 2002) and to enhance TGFβ response in human cells. MED15 also binds to sterol regulatory element binding protein (SREBP), playing a key role in lipid and fatty acid homeostasis (Taubert *et al.* 2006; Yang *et al.* 2006). MED15 thus appears to be a central housekeeping gene, essential for normal development, and largely expressed throughout embryonic stages in metazoans (with purely maternal expression in early stages; Kato *et al.* 2002).

Glutamine repeats, which are common in transcription factors, usually undergo high rates of slippage and rapid evolution (Mularoni 2007). The two sequences identified here may derive from an ancestral neutral polymorphism, with different alleles picked by chance on the X and Y chromosomes when HaMED15 was trapped in the nonrecombining segment. However, the point must also be made that changes in glutamine repeats may have functional consequences (*e.g.*, Gerber

et al. 1994; Shimohata *et al.* 2000; Hancock *et al.* 2001; Buchanan *et al.* 2004). Glutamine chains act as polar zippers, forming hydrogen bonds with complementary polar chains. Changes in repeat numbers may change affinities with specific complementary proteins or induce affinities for other regulatory proteins (Perutz *et al.* 1994). The indels characterized here have thus the potential to modify the conformation of the Mediator complex by changing affinities among the several subunits of its tail module and thereby affect gene transcription in a sex-specific way.

We conclude that the HaMED15 Y allele shows no signs of genetic decay. Whether its differentiation from the X copy reflects the retention of an ancestral neutral polymorphism or the first steps toward the acquisition of a male-specific function remains an open question. A molecular phylogeny of this gene from closely related species might help to determine how long it has been incorporated into a nonrecombining region, to relate the observed differentiation to a divergence time.

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