The **CDY**-related gene family: coordinated evolution in copy number, expression profile and protein sequence

Steve Dorus\(^1,2\), Sandra L. Gilbert\(^1\), Michele L. Forster\(^1\), Robert J. Barndt\(^1\) and Bruce T. Lahn\(^1,\ast\)

\(^1\)Howard Hughes Medical Institute, Department of Human Genetics and \(^2\)Committee on Genetics, University of Chicago, Chicago, IL 60637, USA

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Theories predict that the long-term survival of duplicated genes requires their functional diversification, which can be accomplished by either subfunctionalization (the partitioning of ancestral functions among duplicates) or neofunctionalization (the acquisition of novel function). Here, we characterize the **CDY**-related mammalian gene family, focusing on three aspects of its evolution: gene copy number, tissue expression profile and amino acid sequence. We show that the progenitor of this gene family arose de novo in the mammalian ancestor via domain accretion. This progenitor later duplicated to generate **CDYL** and **CDYL2**, two autosomal genes found in all extant mammals. Prior to human–mouse divergence (and perhaps preceding the eutherian radiation), a processed **CDYL** transcript retroposed onto the Y chromosome to create **CDY**, the Y-linked member of the family. In the simian lineage, **CDY** was retained and subsequently amplified on the Y. In non-simian mammals, however, **CDY** appears to have been lost. The retention of the Y-linked **CDY** genes in simians spurred the process of subfunctionalization and possibly neofunctionalization. Subfunctionalization is evidenced by the observation that simian **CDYL** and **CDYL2** retained their somatic housekeeping transcripts but lost the spermatogenic transcripts to the newly arisen **CDY**. Neofunctionalization is suggested by the rapid evolution of the **CDY** protein sequence. Thus, the **CDY**-related family offers an instructive example of how duplicated genes undergo functional diversification in both expression profile and protein sequence. It also supports the previously postulated notion that there is a tendency for spermatogenic functions to transfer from autosomes to the Y chromosome.

**INTRODUCTION**

According to classical theories, gene duplication creates ‘spare copies’ of genetic material that can be further modified for novel functions (1). Hence, the growth of multigene families through gene duplication has long been recognized as an important fuel for the evolution of biological novelty. At the molecular level, gene duplication can occur by several mechanisms, including genomic transposition, retroposition through an RNA intermediate, uneven crossover between tandem arrays, and whole or partial genome doubling. The classical model of gene family evolution predicts that, following gene duplication, duplicated paralogs will assume one of two principal evolutionary fates (1). The first and more typical one is functional decay, whereby the extra copy degenerates due to functional redundancy whereas the remaining copy maintains its original function. The second and less likely fate is functional diversification, whereby duplicated genes diverge in functional properties. It has been argued that the third possibility—persistence of duplicate genes with identical, redundant functions—is only a transient state, one that cannot be sustained by selection over extended evolutionary periods. Hence, classical theories predict that the productive expansion of gene families in a species will necessarily follow the ‘duplication–diversification’ process.

Subsequent elaboration of the above model led to the notion that functional diversification of duplicated genes can be accomplished in either of two ways (2–4). One is the acquisition of novel function by one or both duplicates (neofunctionalization); the other is the partitioning of the ancestral function among duplicated paralogs (subfunctionalization). A major distinction between neofunctionalization and subfunctionalization lies in the fact that the former is achieved

*To whom correspondence should be addressed. Tel: +1 7738344393; Fax: +1 7738348470; Email: blahn@genetics.uchicago.edu

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by positive selection, whereas the latter is rendered under relaxed selective constraint. A more formalized delineation of subfunctionalization is also known as the ‘duplication–
degeneration–complementation’ (DDC) hypothesis. It postulates that, upon gene duplication, both copies undergo partial degeneration due to relaxation of selection; this results in paralogs whose individual function is diminished relative to the progenitor gene but complementary relative to each other (4). In particular, the DDC hypothesis argues that a likely means of subfunctionalization is the partitioning of the spatio-temporal expression pattern of the progenitor gene between duplicated paralogs. Several examples of such expression partitioning have been reported (4–7), lending credence to the model.

We have previously identified a mammalian multigene family, referred to hereafter as the CDY-related family (8,9). In humans, it consists of many highly related copies on the Y chromosome known collectively as CDY (which stands for chromodomain Y), as well as an autosomal gene known as CDYL (CDY like). In mice, only the autosomal gene, Cdyl, has been identified. Proteins encoded by the CDY-related family are characterized by two functional motifs, a chromodomain implicated in chromatin binding, and a catalytic domain involved in acetylation reactions (8,9). Biochemical data suggest that, consistent with this domain structure, proteins encoded by the gene family possess histone acetyltransferase activity (10). Expression data indicate that the CDY-related family functions in both somatic development and spermatogenesis (9). The spermatogenic function is postulated to be the hyperacetylation of histones during the maturation of spermatids (8,9), which is a prerequisite step for the displacement of histones by the sperm-specific DNA packaging proteins, protamines, at this final stage of spermatogenesis (11,12). In humans, several copies of CDY fall within a region of the Y chromosome frequently deleted in men with azoospermia (no sperm) or oligospermia (very few sperm), suggesting that CDY deletion in humans may contribute to spermatogenic failure (8,13–15).

In this study, we identified a second autosomal member of the CDY-related gene family, which we named CDYL2. We then examined three aspects of the evolutionary history of this family: gene copy number, tissue expression profile and protein sequence. We observed that evolutionary changes in these three aspects are tightly correlated. In particular, we found that expansion of the gene family in copy number has led to significant changes in expression profile and protein sequence. Our results are consistent with the model that gene duplication spurs the process of subfunctionalization and possibly also neofunctionalization. Thus, the story of CDY-related gene family offers an informative example of how duplicated genes evolve toward divergent functionality.

RESULTS

Identification of CDYL2, a new member of the CDY-related family

Previously known members of the CDY-related gene family include multiple, highly related copies of CDY on the human Y chromosome, as well as a single-copy autosomal gene, CDYL, in both human and mouse (8,9). [Given the near identity among all the functional copies of human CDY, all subsequent evolutionary analyses will be based on CDY2, a representative version of the CDY genes (9).] To search for additional members of this gene family, a mouse testis cDNA library was screened with a probe derived from the mouse Cdyl coding sequence. Two classes of clones were uncovered: those that produced strong positive signals at both low and high hybridization stringency, and those that produced signals only at low but not high stringency. The former class of clones corresponded to Cdyl, whereas the latter class belonged to a new member of the CDY-related family. We named this novel member Cdyl2. The mouse Cdyl2 cDNA sequence was then used to obtain the sequence of its human ortholog. Mouse Cdyl2 was mapped using the Jackson Laboratory backcross panel to chromosome 8; human CDYL2 was mapped by a radiation somatic cell hybrid panel to the long arm of chromosome 16, in a region syntenic to the mouse Cdyl2 region (data not shown).

Like other members of the CDY-related family, CDYL2 contains an N-terminal chromodomain and a C-terminal catalytic domain (Fig. 1). Human and mouse CDYL2 genes are highly conserved with each other in amino acid sequence, especially within the chromodomain and the catalytic domain (Fig. 2A). They also share the same intron/exon structure. Human CDYL2 and human CDY have similar but non-identical intron/exon structures, and are quite divergent from each other in their amino acid sequences (Fig. 2B). Finally, human CDYL2 and human CDY genes are even more divergent from each other. It is worth noting that CDYL and CDYL2 are multi-exon genes, whereas CDY is a single-exon gene (Fig. 2B). These data, together with detailed phylogenetic analyses (9) (and see later), indicate that CDYL and CDYL2 arose from genomic duplication of a common progenitor of the gene family, whereas CDY arose some time later from the retroposition of a processed CDYL transcript.

In the course of our work, draft sequences of the human and mouse genomes became available. Searches of these sequences as well as databases of expressed sequence tags (ESTs) failed to identify additional members of the CDY-related family. Given the relative completeness of the human and mouse genome sequences and the comprehensive nature of the EST databases, we think that all members of the gene family in these two species have now been accounted for.

Creation of family progenitor via domain accretion

To understand the evolutionary origin of the CDY-related family, we searched for its homologs in distantly related species. The two protein motifs encoded by this gene family were individually found in many other species. The chromodomain must be as old as eukaryotes since it is present in various chromatin-binding proteins of virtually all eukaryotes, such as yeast Swi6, tetrahymena PDD1, Drosophila Polycomb and mammalian HP1. The catalytic domain is even more ancient, as it is found in numerous metabolic enzymes of both eukaryotes and prokaryotes. Interestingly, the combination of these two ancient domains within the same gene is a unique characteristic of the CDY-related family; we have thus far failed to identify any non-mammalian genes that contain both domains. The most plausible interpretation of this observation
is that the progenitor of the CDY-related family arose de novo by domain accretion in the lineage leading to modern mammals. One likely mechanism is exon shuffling (16), whereby exons encoding the chromodomain are juxtaposed in front of exons encoding the catalytic domain via some type of genomic rearrangement such as translocation or transposition.

Expansion of the gene family

To examine the evolutionary history of the CDY-related family, we constructed a phylogenetic tree using the deduced protein sequences of CDY, CDYL, and CDYL2 in both human and mouse (Fig. 3). This tree revealed that CDYL and CDYL2 descended from a common progenitor through an ancient duplication, whereas CDY arose much more recently as a derivative of CDYL. Because all copies of CDY on the human Y chromosome are single-exon genes, the most likely mechanism by which CDY arose de novo on the Y chromosome is the retro-position of a processed CDYL transcript (9). This retroposition event was apparently followed by several rounds of amplification to create multiple copies of the gene on the present-day human Y chromosome. Similar amplifications of CDY on the Y appear to have occurred in other simian lineages as well (unpublished data).

We have previously proposed that CDY arose de novo during primate evolution, in the common ancestor of simians and after the simian-prosimian split (9). This model was based on the observation that CDY can only be found in simian primates (9). However, as shown in Figure 3, the phylogenetic relationship among members of the CDY-related family is actually more consistent with human CDY having arisen prior to human–mouse divergence (and perhaps even predating the eutherian radiation), which is much earlier than that assumed by our previous model (9). One possibility could be that the tree in Figure 3 is erroneous due to accelerated protein evolution of CDY (indeed, faster evolution of CDY is readily visible in Fig. 3). To address this possibility, we used the highly conserved chromo and catalytic domains to more reliably deduce the phylogenetic relationship among members of the CDY-related family. This led to phylogenetic trees with very similar topology to that shown in Figure 3 (data not shown), supporting our model of an early CDY creation prior to human–mouse divergence. We therefore propose that CDY probably arose early during mammalian evolution, persisting only in the simian lineage but lost in most (if not all) other mammals.

There are informative evolutionary parallels (and contrasts) between the CDY-related family and the DAZ-related family that also indicate a more ancient creation of CDY. The latter gene family consists of multiple, highly related copies of the DAZ genes on the human Y chromosome, and a single autosomal gene, DAZL (17,18). Similar to the evolutionary relationship between CDY and CDYL, DAZ is believed to have arisen on the Y chromosome de novo as a copy of the more ancestral DAZL gene, and to have subsequently amplified on the Y (18). However, DAZ apparently arose by genomic transposition
(rather than by retroposition as is the case for CDY). Additionally, DAZ is believed to have arisen very recently during primate evolution, in the common ancestor of Old World monkeys and apes, but after the split between Old World and New World monkeys (18). If the emergence of CDY is indeed much earlier than DAZ, one would predict that CDY should be much more distantly related to CDYL than DAZ is to DAZL. To test this prediction, we analyzed the evolutionary divergence among human CDY, human and mouse CDYL, and the macaque monkey CDY (which we cloned and sequenced).

We then compared the result with the evolutionary divergence among human DAZ, human and mouse DAZL, and macaque DAZL (Fig. 4). Here, we used the rate of synonymous substitutions ($K_s$) as a measure for evolutionary time. As shown in Figure 4, the $K_s$ of CDY between human and macaque is virtually identical to the $K_s$ of DAZ between these two species, as one would expect. In contrast, the $K_s$ between human CDY and human CDYL is many-fold greater than the $K_s$
between human DAZ and human DAZL (Fig. 4). This observation provides further evidence that, contrary to our previous model (9), the creation of CDY was a very ancient event that happened in the early history of mammals.

**Evolution of expression profile is consistent with subfunctionalization**

According to the classical model, duplicated genes will necessarily undergo functional diversification; otherwise, one of the duplicates will eventually decay due to redundancy (1). In theory, there are two mechanisms by which duplicated genes can diversify in function: they can either evolve different spatio-temporal expression patterns, or acquire distinct protein functions through coding sequence changes. We wished to first examine the evolution of the CDY-related family with respect to its tissue expression profile. By combining current and previous data (9), we obtained expression patterns of the gene family in three species: mouse, rabbit and human (Fig. 5). In mouse and rabbit, both CDYL and CDYL2 express a ubiquitous long transcript as well as a highly abundant testis-specific short transcript. In human, the CDYL and CDYL2 genes express the ubiquitous long transcript just as they do in the mouse and rabbit. However, they have essentially lost the testis-specific short transcript. Importantly, the Y-linked CDY genes in human are expressed exclusively and abundantly in the testis. Thus, the combined tissue expression patterns of CDY, CDYL and CDYL2 in human recapitulate the expression patterns of CDY and CDYL2 in mouse or rabbit. As is typical for non-simian mammals (9), mouse and rabbit only have the autosomal CDYL and CDYL2 genes. By contrast, human possesses the Y-linked CDY genes in addition to the two autosomal genes. Thus, there is a correlation between the gene composition of the CDY-related family in a species and the expression profile of the family in that species.

These observations are consistent with subfunctionalization of duplicated genes by the duplication-degeneration-complementation process (4). Specifically, we propose that, prior to the emergence of CDY, the two autosomal genes, CDYL and CDYL2, expressed the ubiquitous long transcripts with somatic housekeeping function, as well as the highly abundant testis-specific short transcripts with spermatogenic function. This state of gene expression and function has remained in extant mammals lacking CDY. Indeed, studies of mouse CDYL are consistent with its having distinct somatic and spermatogenic functions (10) (unpublished data). In simians where the newly arisen CDY persisted and amplified, the two distinct transcripts (and presumably the two functions) of the ancestral autosomal genes were partitioned. The autosomal genes have retained their ubiquitous transcripts but have lost the abundant testis transcripts. As a complement, the Y-linked CDY genes have acquired a testis-specific expression pattern. One aspect of this model requires further consideration. If CDY was indeed present in all early mammals but was subsequently lost in all but the simian lineage, what was the expression status of CDY, CDYL and CDYL2 in early mammals before and after the loss of CDY? There are two possibilities. First, the testis transcript was transferred from CDYL and CDYL2 to CDY in early mammals, but upon the loss of CDY in non-simians, CDYL and CDYL2 re-acquired the testis transcript. Alternatively, CDY, CDYL and CDYL2 in early mammals all expressed the testis transcript, but in the simian lineage where CDY persisted to the present day, the CDYL and CDYL2 genes eventually lost the testis transcript. We favor the latter scenario because it is more parsimonious, but we cannot rule out the former possibility.

**Evolution of coding sequence suggests neofunctionalization**

In addition to alterations of expression profile, functional diversification of duplicated genes can also result from modifications of protein sequence. This appears to be the case for the Y-linked CDY. The CDY branch in the protein phylogeny of the gene family is significantly extended relative to the other branches (Fig. 3), suggesting that protein sequence of CDY has evolved much more rapidly than CDYL and CDYL2. To confirm this apparent acceleration of CDY protein sequence evolution, we compared all members of the gene family between human and the macaque monkey (for this, we cloned and sequenced the relevant macaque genes). For each gene, we then calculated the ratio of the human–macaque non-synonymous substitution rate ($K_a$) to the corresponding synonymous substitution rate ($K_s$). This ratio measures the rate of protein sequence evolution scaled to the neutral mutation rate. As shown in Figure 6, the human–macaque $K_a/K_s$ ratio for CDY is significantly higher—by many fold—than that for CDYL and CDYL2. This demonstrates unequivocally that protein evolution of CDY is greatly accelerated relative to the other members of the gene family. Such an observation is consistent with the notion that CDY might have undergone functional diversification from its autosomal paralogs through protein sequence modifications.
To further substantiate this finding, we sequenced \( CDY \) in the chimpanzee. As shown in Figure 6, the \( K_s/K_a \) ratio of \( CDY \) between human and chimpanzee is even higher than that between human and macaque. In fact, this ratio is greater than 1, which suggests that the rapid protein evolution of \( CDY \) may be driven by positive selection.

**DISCUSSION**

In this study, we reconstructed the evolutionary history of the \( CDY \)-related mammalian gene family. The progenitor of the family arose in the early ancestor of mammals. The mechanism responsible for the creation of this progenitor is most likely domain accretion by exon shuffling, whereby a region encoding the chromodomain was juxtaposed by genomic rearrangement in front of another region encoding the catalytic domain.
Thereafter, multiple duplication events transpired to expand the gene family. The first event took place in the common ancestor of mammals, whereby a single genomic duplication resulted in two autosomal genes, CDYL and CDYL2, both of which persisted in all extant mammalian species. The second event occurred prior to human–mouse divergence (and perhaps predating the eutherian radiation), whereby a processed CDYL transcript was retroposed onto the Y chromosome. This retroposed gene was retained in the simian lineage where it subsequently amplified further into multiple copies. However, it appears to have been lost in most (if not all) other mammals.

The classical model of genome evolution posits that the growth of multigene families proceeds along a characteristic path, starting with gene duplication and followed by functional diversification of duplicated copies (1). Functional diversification of duplicated genes can be accomplished through either the acquisition of novel function by one or more of the duplicates (neofunctionalization), or the partitioning of the ancestral function among duplicates (subfunctionalization) (3,4). The process of subfunctionalization was further formalized into the ‘duplication–degeneration–complementation’ (DDC) hypothesis, which argues that duplicated genes can each undergo partial degeneration to become functionally complementary to each other (4–7).

The evolution of the CDY-related family offers an instructive example of subfunctionalization by the DDC process. Our results support the notion that in mammalian ancestors, the two autosomal members of the gene family, CDYL and CDYL2, each expressed a ubiquitous long transcript with somatic housekeeping function, and a highly abundant testis-specific short transcript with spermatogenic function. In the non-simian mammals examined (which lack CDY), this ancestral expression profile persisted. In humans, CDYL and CDYL2 retained the ubiquitous transcripts, but lost the testis-specific transcripts to the Y-linked CDY. Clearly, the process of subfunctionalization in the evolution of the CDY-related family is accomplished through changes in tissue expression patterns. It is not clear how CDY acquired its testis-specific expression. Given that this gene arose by retroposition, it presumably did not carry any promoter sequences with it when it first inserted into the Y chromosome. One possibility is that it landed behind a testis-specific promoter on the Y, and thus acquired testis-specific expression from the beginning. Alternatively, it could have initially landed in a location that conferred little or non-specific expression, but, over time, evolved testis expression under selection. Once CDY arose and acquired testis expression, CDYL or CDYL2 presumably accumulated mutations in their promoters that resulted in the loss of their testis-specific transcripts.

While the evolution of the gene family’s expression pattern is consistent with subfunctionalization, sequence evolution of the family is suggestive of neofunctionalization. Specifically, the significantly accelerated rate of CDY protein evolution relative to CDYL and CDYL2 suggests that CDY may have evolved under positive selection. Thus, both subfunctionalization and subsequent neofunctionalization appear to have affected the evolution of the CDY-related family.

The non-recombining portion of the Y chromosome is the only segment of the genome that evolves exclusively in a male context. It is believed that due to this unique property, the Y may act as an ‘attractor’ for genes that benefit males but are inconsequential or harmful to females (8,9,18,19). Genes functioning specifically in spermatogenesis surely fall into this category. Indeed, of the small number of genes that persisted on the modern Y, a disproportionate fraction appears to encode testis-specific genes with putative spermatogenic functions (8). Thus, the transfer of spermatogenic function from the ancestral autosomal CDYL and CDYL2 genes to the Y-chromosomal CDY may be more than chance happenstance. Rather, there may be a selective advantage in moving the spermatogenic function of the gene family from autosomal locations (which are shared equally between males and females) to the male-specific Y chromosome.

Male-reproductive genes, including those involved in spermatogenesis, are known to evolve exceedingly fast, presumably as a result of intense sexual selection (20). The rapid evolution of CDY, which is expressed testis-specifically, may also be the result of sexual selection. Indeed, given the putative function of CDY in promoting chromatin remodeling during spermatogenesis (10), it is conceivable that changes in CDY protein sequence could affect the developmental and physiological properties of sperm. As such, the rapid evolution of CDY protein sequence may be the result of positive Darwinian selection conferred by sperm competition or other forms of sexual selection. In species that lack CDY, the autosomal CDYL and CDYL2 carry the somatic housekeeping function in addition to the spermatogenic function. Owing to such dual responsibility, these genes are perhaps under much greater functional constraint, and are therefore not free to evolve rapidly under sexual selection as is the case for CDY. So, while many duplicated genes may appear to have undergone subfunctionalization based on the partitioning of expression patterns (4–7), the more circumscribed expression of each duplicated paralog relative to the progenitor may free it to better respond to positive selection that ultimately optimizes/improves its function. The result is some degree of neofunctionalization following subfunctionalization. We argue, therefore, that in the course of gene family growth, subfunctionalization may be coupled to (and provide a trigger for) neofunctionalization.

MATERIALS AND METHODS

Cloning and mapping of human and mouse CDYL2

A partial clone of murine Cdyl2 was isolated by a hybridization screen of an adult BALB/c mouse testes cDNA phage library from Clontech (Palo Alto, CA, USA), utilizing mouse Cdyl coding sequence as a probe. The remainder of the cDNA sequence was obtained by 5' and 3' RACE (rapid amplification of cDNA ends) of adult murine testis and brain cDNA pools (Clontech). Complete cDNA sequence of human CDYL2 was identified by a combination of database search that retrieved the human clone RP11-18F14 (GenBank accession number: AC018956.4), and 5' and 3' RACE on human brain, testis and prostate cDNA pools. Murine Cdyl2 was mapped to the distal arm of chromosome 8 using the Jackson BSS backcross panel (information available at www.informatics.jax.org/searches/crossdata_form.shtml); human CDYL2 was mapped
to the long arm of chromosome 16 using the GeneBridge 4 radiation hybrid panel (21). GenBank accession numbers for human CDYL2 and murine Cdyl2 are AY273798 and AY273799, respectively. Accession numbers of additional sequences utilized in this study are: human CDY2 (NM_004825), human CDYL-major isoform (NM_170723), human CDYL-minor isoform (NM_004680), mouse Cdyl (NM_009881), human DAZ (XM_088763), human DAZL (NM_001351) and mouse Dazl (NM_0010021).

Cloning of macaque CDY, CDYL and CDYL2

The crab-eating macaque monkey (cynomolgus monkey; Macaca fascicularis) cDNA sequence of CDYL and CDYL2 was obtained by PCR (polymerase chain reaction) from pools of cDNA generated from equal amounts of mRNA isolated from heart, brain, kidney, muscle, testis and lung samples. The cDNA sequences of macaque and chimpanzee CDY were similarly obtained from testis cDNAs of the respective species. PCR primers were designed based on sequences of the human genes. All sequencing was done using the dye-terminator PCR primers were designed based on sequences of the human genes. All sequencing was done using the dye-terminator method on the ABI 3700 capillary sequencer (PE Applied Biosystems). All sequencing was done using the dye-terminator method on the ABI 3700 capillary sequencer (PE Applied Biosystems, Foster City, CA, USA). GenBank accession numbers for macaque CDY, CDYL and CDYL2, and for chimpanzee CDY are AJ314841, AY275460, AY271718 and AY292530, respectively.

Northern analysis

The rabbit multiple-tissue northern blots were generated by the standard protocol, using 10 μg of total RNA for each tissue. The human and mouse blots were purchased from Clontech, which contained 2 μg of mRNA for each tissue. The CDY and CDYL northern data for human and mouse were as previously published (9).

Phylogenetic analysis

Phylogenies of the CDY and DAZ gene families were constructed using a combination of software packages, including MegAlign (DNASTAR, Madison, WI, USA), PAUP (Sinauer Associates, Sunderland, MA, USA) and GCG (Genetics Computing Group, Madison, WI, USA). The Ks and K values between homologs were obtained utilizing the Diverge function in GCG (22). In calculating divergence between members of the CDY-related family, only the beginning of the chromodomain to the end of the coding sequence was used, because coding sequences before the chromodomain are often not alignable between family members. The alignment of DAZ between human and macaque only utilized the first of the repeated RNA binding domains, due to a dramatic expansion of the repeats in the macaque.

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REFERENCES