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DAX-1, an 'antitestis' gene

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Abstract. The *DAX-1* gene has been involved in the dosage sensitive sex reversal (DSS) phenotype, a male-to-female sex-reversal syndrome due to the duplication of a small region of human chromosome Xp21. *Dax-1* and *Sry* have been shown to act antagonistically in the mouse system, where increasing expression of the former leads to female development and increasing activity of the latter to male development. Although these data strongly implicate *DAX-1* in sex determination, the mouse and human proteins appear to behave differently. Absence of DAX-1 is responsible for adrenal hypoplasia congenita, a human inherited disorder characterized by adrenal insufficiency and hypogonadotropic hypogonadism. Unlike human patients, *Dax-1*-deficient XY mice have normal levels of corticotropins and adrenal hormones but are sterile. *Dax-1*-deficient females are fertile. The DAX-1 protein, an unusual member of the nuclear hormone receptor, may act as a transcriptional repressor. It has been shown to both repress transcriptional activators by direct protein-protein interactions and to bind DNA hairpin structures and repress target genes.

Key words. *DAX-1*; AHC; sex determination; male-to-female sex reversal; adrenal development.

DAX-1 function

DAX-1 and the DSS phenotype

The *DAX-1* gene [1] was isolated from the DSS (dosage sensitive sex reversal) locus, a region of the short arm of the human X chromosome (Xp21) [2]. XY individuals, with a duplication of DSS, exhibit male-to-female sex reversal and gonadal dysgenesis, despite the presence of an intact *SRY* gene. Although XXY individuals develop as males, the single X chromosome in patients with DSS duplications does not undergo X inactivation, implying that the sex-reversal gene is both dosage-sensitive and normally subject to X inactivation. XY patients with deletions of the entire DSS region develop as males [2]. Taken together, these observations suggest that a gene(s) within the DSS region disrupts testis formation when present in a double dose but it is not required for normal testis development. It was proposed that this gene (or genes) is an 'antitestis gene' that may be required for ovarian development [2].

The smallest duplication, found in sex-reversed patients, is a 160-kb region of Xp21. This region contains, in addition to the *DAX-1* gene, the *MAGEb/DAM* genes: a family of four to five genes related to the *MAGE* gene family. *MAGE* genes encode tumour-associated antigens of unknown function [3, 4]. As described below, recent biochemical and genetic experiments have equated *DAX-1* with the sex-reversal gene DSS.

Dax-1 overexpression in the mouse

The hypothesis that *DAX-1* is equivalent to DSS was tested by overexpression of *Dax-1* (the murine homologue of *DAX-1*) in transgenic mice. Mice were constructed with extra copies of the *Dax-1* gene [5]. High levels of exogenous *Dax-1* expression in the genital ridge retards testis differentiation in XY mice with an *Sry* allele of *Mus musculus musculus* origin, but complete sex reversal occurred when other alleles of *Sry* were used, either the naturally occurring *M. poschiavinus* allele or an *Sry* transgene. This supports the hypothesis that *Dax-1* and *Sry* act antagonistically, where increasing expression of the former leads to female

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development and increasing activity of the latter to male development.

Although these data strongly implicate *Dax-1* in sex determination, the mouse and human systems appear to be behaving differently. A double dose of human *DAX-1* is sufficient to sex-reverse patients with duplications of Xp21, but much higher levels of expression seem to be required for the mouse *Dax-1* gene to compete effectively with an *Sry* allele of *M. m. musculus* origin. One explanation would be the existence of an additional gene(s) in the 160-kb minimal DSS region that cooperates with *DAX-1* to achieve complete sex reversal when present in double dose. A more plausible explanation is that *Sry* and *Dax-1* show species differences in levels of expression relative to critical thresholds. For example, the *M. m. musculus Sry* gene could be expressed significantly higher than the minimum level required to induce testes. Perhaps the human *SRY* gene is similar to the *M. poschiavinus Sry* gene that appears to be expressed at a level much closer to its threshold.

Another possible species difference is in the precise timing of expression of the two genes. If *Dax-1* expression is delayed with respect to *Sry* in *M. m. musculus*, it may never reach the level required to compete before *Sry* has reached its critical threshold and initiated Sertoli cell differentiation. If *Sry* expression is delayed, high levels of *Dax-1* would be able to act first. Testis formation in transgenic XX *Dax*:*Sry* embryos is retarded in comparison to that in normal XY embryos. This is consistent with a delay in *Sry* expression when it is driven by the *Dax-1* regulatory region and may explain why *Dax-1* competes effectively with *Sry* when the two genes are expressed from identical regulatory sequences.

Finally, subsequent events in gonadal differentiation may differ between humans and mice. In the mouse, fetal ovo-testes usually resolve into mature testes by birth. Perhaps in humans, the bias is towards maintaining ovarian (or streak) development. If this were the case, more examples of sex reversal would be detected in humans than mice.

DAX-1 absence

DAX-1 functional absence in XY individuals. Absence of a functional DAX-1 protein is responsible for the X-linked form of adrenal hypoplasia congenita (AHC) [1, 6]. AHC (MIM 300200, http://www.ncbi.nlm.nih.gov/omim) is an inherited disorder of adrenal gland development, characterized by marked underdevelopment or absence of the permanent zone of the adrenal cortex and by structural disorganization of both the fetal cortex and the adult glands. The disorder, which is lethal if untreated, results in adrenal insufficiency early in infancy, with low serum concentrations of glucocorticoids, mineralcorticoids and androgens, and failure to respond to ACTH stimulation. Hypogonadotropic hypogonadism (HH) is commonly associated with the X-linked form of the disease and is generally diagnosed at the expected time of puberty. Clinical investigations of AHC patients provided conflicting results regarding hypothalamic or pituitary origin of the etiology of HH [7]. Expression studies have demonstrated that *DAX-1* messenger RNA (mRNA) is present in both the pituitary gland and in the hypothalamus (see below), consistent with a direct role for DAX-1 at both levels.

Many deletions and point mutations in the *DAX-1* gene have been described in AHC patients (reviewed in [8, 9]). Most point mutations (42 out of 49) are frameshift or nonsense mutations that introduce a premature stop codon and are distributed throughout the whole length of the transcript. All the mutations that cause single amino acid changes (6 missense and a single one-codon deletion) alter conserved amino acids in the ligand binding domain (LBD, see fig. 1). Zhang et al. [8] have constructed a model of the DAX-1 LBD by homology to the three-dimensional crystal structure of the thyroid and retinoid X nuclear hormone receptors. The missense mutations and the codon deletion in DAX-1 all mapped to the predicted hydrophobic core of the LBD.

Clinical heterogeneity has been described between AHC patients, with some requiring hormonal replacement therapy within the first weeks after birth and others not being diagnosed until after 5 years of age. The variation in clinical presentation does not correlate with the extent of the molecular lesion and has even been described between members of the same AHC family.

Dax-1 functional absence in XY mice. A targeted disruption of *Dax-1* has recently been achieved by Yu et al. [10], using a conditional Cre-loxP approach. This strategy was needed because *Dax-1* was found to be essential for embryonic stem cell growth and differentiation. The disrupted allele lacks the second exon and mimics mutations found in AHC patients. Cre-mediated excision of the second exon also ablates the intronic splice acceptor site and the polyadenylation signal, resulting in low levels of an abnormal, unspliced transcript [10].

Unlike human AHC patients, Dax-1-deficient XY mice (AHC mice) are indistinguishable from their wild-type littermates until sexual maturation. Serum corticosterone levels in mutants are similar to those of wild-type animals. Histological inspection of the adrenals suggests that Dax-1 function is required for the initiation of fetal adrenal degeneration (which occurs after puberty), but is not necessary either for the formation of the definitive cortex or for steroidogenesis. Testosterone production during embryonic and early postnatal development is sufficient for the formation of male internal and exter-

nal genitalia, for testicular descent (most AHC human patients show cryptorchidism) and for the normal development of the testosterone-sensitive seminal vesicles. AHC mice are hypogonadal, but unlike AHC human patients their gonadotropin levels are normal, suggesting primary testicular failure. Testicular weights are approximately one half of those of wild-type animals. Dax-1 deficiency causes progressive degeneration of the testicular germinal epithelium and results in sterility.

DAX-1 functional absence in XX individuals. As expected in X-linked recessive disorders, heterozygous carriers of AHC mutations are normal. The occurrence of homozygous DAX-1-deficient human females is unlikely because males, who would be needed to transmit the nonfunctional allele, are sterile.

Dax-1 functional absence in XX mice. The disruption of *Dax-1* in female mice does not appear to prevent sexual maturation, ovulation or fertility [10]. Histological analyses of ovaries from mature females show a normal complement of follicles at different stages of maturation as well as the presence of corpora lutea. Dax-1-deficient females mated with wild-type males produce normal litter sizes with equal transmission of the mutation to male and female offspring. The only abnormality found in mutant females is the presence of multiple oocytes in a subset of follicles. A single thecal layer is present and surrounds the proliferating granulosa cell layer and oocytes, implicating Dax-1 in follicular recruitment, granulosa cell proliferation or in the formation of structures that segregate different follicles.

Species differences in DAX-1 function

The comparison of natural human mutations and murine models reveals several apparent differences in the effect of DAX-1 dosage in humans and mice. A possible explanation of the dichotomy is that the murine models do not reflect accurately the physiological situation. The promoter sequences used for transgenic overexpression experiments could lack specific sequences required for precise timing of expression. In the targeted disruption experiments, the disrupted allele may encode a protein with residual biological activity. Alternatively, the observed species differences may reflect species-specific changes in the processes that control adrenal and gonadal development and function. Accordingly, the high degree of divergence between human and mouse DAX-1 proteins (see below) may suggest that they act in the context of a rapidly evolving system.

DAX-1 structure

The *DAX-1* gene encodes an unusual member of the nuclear hormone receptor (NR) superfamily. The protein is between 470 and 474 amino acids in length in the different mammalian species analysed to date [1, 11–14]. The DAX-1 protein can be divided into two parts with different structural and functional features (fig. 1).

The carboxy-terminal half of the protein is similar to the ligand binding domain (LBD) of the NR superfamily. The highest homology is observed with SHP

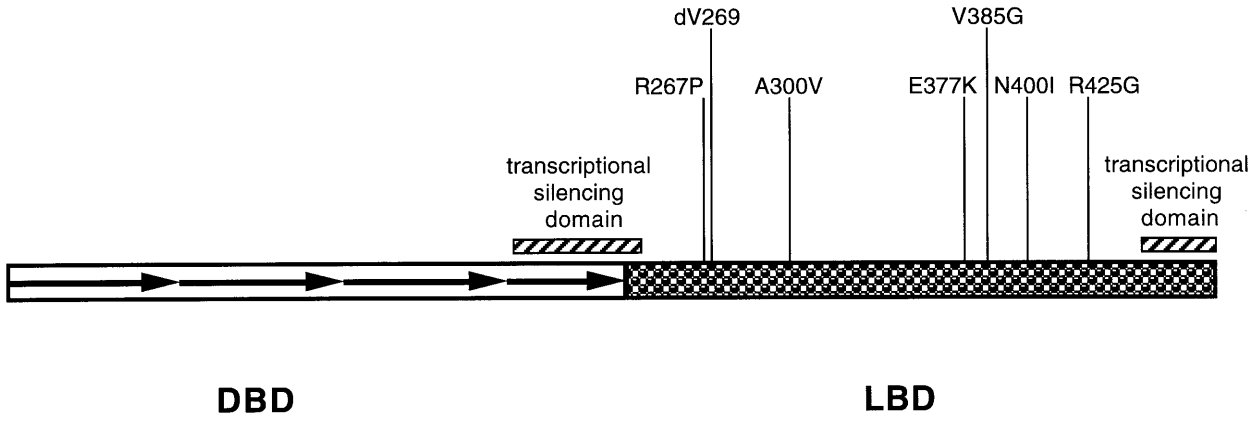

Figure 1. Schematic representation of the DAX-1 protein showing the putative DNA binding and ligand binding domains (DBD and LBD, respectively) and the transcriptional silencing domains. The AHC mutations that cause a single amino acid change are also shown.

(an unusual member of the NR family that contains a canonical LBD but no DBD) [15], with the RXR and the orphan (see [1] for references) receptor subfamilies. The level of homology between DAX-1 and the other members of the NR superfamily, although significant, does not indicate the nature of the hypothetical ligand. A remarkable feature of the human DAX-1 LBD is the presence of an unusually long insertion which is in the proximity of the predicted ligand binding pocket. This region is poorly conserved between species and may imply the absence of a specific ligand for DAX-1 (fig. 1). The most conserved portions of the LBD contain the transcriptional silencing domains (see below).

The DNA binding domain (DBD) of NR proteins is composed of two zinc fingers and is conserved amongst the different types of receptors. In DAX-1 the canonical DBD of NR proteins is missing and is substituted by a novel N-terminal repetitive structure, organized into four incomplete repetitions of an alanine- and glycine-rich 65–67-amino acid motif. The repeats, showing 33–70% identity to each other, contain conserved cysteine residues. This N-terminal structure of DAX-1 shows no obvious similarity to previously reported protein sequences, and might define a novel DNA binding domain with specificity for DNA hairpin structures [16].

Lalli et al. [17] have compared the structure of the LBD of human and mouse DAX-1 to the three-dimensional structure of the LBD of apo-RXRα and holo-RARγ. They identified in DAX-1 the domains corresponding to α-helices 1–12, which represent the hallmark of the NR LBD. Their analysis also suggested that helix H1 of the DAX-1 LBD encompasses the region previously defined as the last and incomplete DBD repeat.

NRs are highly conserved between different species, with LBDs showing continuous similarity levels higher than 85%. Detailed comparison of human and mouse DAX-1 sequences [12] reveals subdomains where the predicted proteins are highly conserved (81–96% identity and 90–100% similarity) separated by amino acid stretches, accounting for approximately half of the protein, which are only poorly conserved (41–55% identity and 58–71% similarity). The protein-coding sequences of *DAX-1* and *Dax-1* are more similar at the DNA than at the protein level, suggesting rapid evolution. This pattern of evolution might be accounted for either by a process of random fixation of neutral point mutations in subregions lacking functional constraints or by positive selection for divergence. Positive selection is suggested by the very high nonsynonymous to synonymous substitution ($K_a/K_s > 0.8$) ratio in the central portion of the LBD. It is worth noting that *SRY* is also a rapidly evolving gene, whereas other genes involved in sex determination and gonadal differentiation such as *SOX9*, *WT1* and *SF-1* (see accompanying reviews) are highly conserved between mouse and humans. This type of rapid evolution could reflect competition between SRY and DAX-1 and be related to their positions at the top of a hierarchy.

DAX-1 pattern of expression

The phenotypic similarities that accompany disruption of the *Sf-1* gene (see accompanying review) and mutations in *DAX-1* suggest that these two genes act in the same developmental pathways, and in situ hybridization studies suggest a striking colocalization of the mRNAs for *Dax-1* and *Sf-1* within four developing organs: adrenals, gonads, hypothalamus and pituitary gland [12, 18, 19]. Together these results are consistent with DAX-1 and Sf-1 determining adrenal and gonadal development and modulating reproductive function at hypothalamic, pituitary and gonadal levels.

Dax-1 is expressed in the adrenals from the very earliest stages of their development and may be expressed within the cells making up the lineage that gives rise to adrenal cells prior to their separation from the genital ridge. The gene is then expressed at all stages of adrenal development including the adult organ. *Dax-1* expression is always restricted to the adrenal cortex [12, 19].

AHC human patients, but not *Dax-1* knockout mice, are also characterized by HH. *Dax-1* is expressed in the developing hypothalamus, and in the pituitary at low levels, which implies that the HH phenotype seen in AHC patients may be due directly to DAX-1 functional deficiency [12, 19]. By 11.5 dpc, *Dax-1* expression is detected in the same region of the developing brain that expresses *Sf-1*. By 14.5 dpc, expression has localized to the retrochiasmatic ventral diencephalon, which ultimately contributes to the hypothalamus, and by 18.5 dpc to the ventromedial hypothalamic nucleus, the site were both *Sf-1* and *Dax-1* are expressed in the newborn and adult mouse brain [19]. The anterior pituitary gland contains five discrete cell types, of which only the gonadotropes express *Sf-1* and *Dax-1* [19].

The onset of *Dax-1* expression in both XX and XY genital ridges occurs at the same time as *Sry* in XY genital ridges. After 12.5 dpc, when *Sry* expression disappears, *Dax-1* expression in the testis decreases dramatically while it persists in the ovary. This supports the proposal that *Dax-1* interferes with testis development and that its expression must be repressed in testis.

Dax-1 positive cells show a distribution within the developing gonads that has not been observed previously. One interpretation of this pattern is that *Dax-1* marks a specific stage of relatively undifferentiated cells. These cells disappear rapidly in the testis, but remain longer in the ovary, especially in a region adjacent to the mesonephros. This distribution pattern could be used to argue that Sry initiates differentiation of the

genital ridge into a testis which in turn represses *Dax-1* expression. The group of cells in the anterior region of the gonad expressing *Dax-1* in both sexes could correspond either to cells which have escaped the signal to differentiate or to a remnant of adrenal precursors [5, 12].

In the adult mouse, *Dax-1* is primarily expressed in steroidogenic tissues, including the adrenal cortex, testicular Leydig cells and ovarian thecal and granulosa cells [12, 19]. Expression in rat Sertoli cells was reported by Tamai et al. [20], who first suggested that Dax-1 may influence the development of spermatogenic cells in response to steroid and pituitary hormones. The expression is regulated during spermatogenesis and peaks during the androgen-sensitive phase of the spermatogenic cycle. Maximum levels are present between postnatal days 20 and 30 in the rat, during the first spermatogenic wave. Treatment of cultured Sertoli cells with follicle stimulating hormone (FSH), an important regulator of spermatogenesis, results in a potent down-regulation of *Dax-1* expression [20]. These data fit with the recent finding that Dax-1-deficient male mice are sterile due to progressive epithelial dysgenesis with complete loss of germ cells by 14 weeks [10].

Regulation of the expression of *DAX-1*

Does *SF-1* regulate *DAX-1*?

The overlapping tissue distributions and known roles of SF-1 and DAX-1 raise the possibility that they interact either directly or indirectly. An obvious possibility is that the receptors act in a developmental cascade and one factor regulates the expression of the other gene. The 5′-flanking region of the human and mouse *DAX-1* genes contain a sequence that matches the consensus DNA binding motif for SF-1 protein; this sequence binds SF-1 in gel mobility shift assays (EMSA) [19, 21]. However, 5′ deletion analyses were used to show that the putative SF-1-responsive element does not regulate expression of *Dax-1* either in mouse Y-1 adrenocortical cells (a cell line that does not express *Dax-1* endogenously) or in *Dax-1*-expressing MA-10 Leydig [19]. In addition, *Dax-1* expression persists in the gonads and the hypothalamus of a mouse with a disrupted *Sf-1* gene [19]. As suggested by Ikeda et al. [19], these findings imply that Sf-1 is not an obligatory positive regulator of *Dax-1*.

In a more recent set of experiments, Yu et al. [22] discovered that the murine *Dax-1* promoter contains a duplicated binding site for Sf-1. These sites are not completely conserved in the human gene. The combined sites (-134 to -114 in the murine promoter) function as a composite element that is capable of interacting with several proteins in the nuclear extracts from αT3, a gonadotrope cell line that expresses both *Dax-1* and *Sf-1*, and Y-1, an adrenocortical cell line that expresses *Sf-1* but not *Dax-1*. In both cell lines, the formation of three major complexes was observed, and supershift analyses identified two of these complexes as containing Sf-1 and COUP-TF. Both half sites were required for full Sf-1-mediated activation: disruption of these sites reduced by half the basal activity of the native *Dax-1* promoter in both αT3 and Y-1 cells. Disruption of the first site increased basal promoter activity in a placental choriocarcinoma cell line, JEG-3, that does not usually express *Dax-1* and *Sf-1*. These results suggest the existence of a repressor protein capable of interacting at this site. When linked to the minimal thymidine kinase promoter, each of the isolated Sf-1 sites was sufficient to mediate transcriptional regulation by Sf-1. Yu et al. [22] proposed that *Dax-1* expression is stimulated by Sf-1, and that Sf-1 and COUP-TF provide antagonistic pathways that converge upon a common regulatory site.

Does DAX-1 repress its own expression?

The observation that Dax-1 can bind to hairpin DNA structures (see below) prompted a search for such structures in gene promoters likely to be under Dax-1 regulation. Zazopoulos et al. [16] detected two possible hairpins (H1 and H2) in the promoter of the murine *Dax-1* gene and showed that H1 formation is favoured over H2. In transient transfection experiments, in COS-1 cells, using a reporter gene carrying the luciferase gene under the control of *Dax-1* promoter sequences, Dax-1 protein moderately represses the basal activity of both wild-type and H1-deleted promoters, whereas it drastically blocks Sf-1-mediated activation of the wild-type but not H1-deleted *Dax-1* promoter. It was suggested that the proximity of Sf-1 and Dax-1 binding sites in the *Dax-1* promoters may allow an allosteric inhibition of Sf-1 binding (see below) [16].

The promoter defined by transgenic experiments

An 11-kb genomic fragment, derived from the region immediately upstream of the *Dax-1* start of transcription, is capable of targeting expression of a β-galactosidase reporter gene in the developing gonads in a pattern identical to the endogenous *Dax-1* gene [5]. In the transgenic animals, β-galactosidase activity was first observed at 10.5 dpc within the genital ridge, and reached its highest level by 11.5 dpc in both male and female embryos. This activity persists throughout ovary formation, but in the male it is rapidly downregulated around 12.5 dpc, although it is maintained in the region between the gonad and mesonephros in a manner identical to the endogenous gene. In the adult animals, reporter gene expression also followed that of the endogenous gene in gonadal sites. Interestingly, β-galac-

tosidase activity was absent from other sites of endogenous *Dax-1* expression, such as the developing adrenal, hypothalamus and pituitary, suggesting that additional upstream sequences are necessary to obtain the complete pattern of expression of this gene [5].

DAX-1 acts as a potent transcriptional repressor

Several groups have reported that DAX-1 acts, in vitro and in vivo, as a potent transcriptional repressor. DAX-1 inhibits SF-1-mediated transactivation of target genes and antagonizes SF-1/WT1 synergy—probably through a direct interaction with SF-1. Dax-1 also represses *StAR* (steroidogenic acute regulatory protein) gene expression.

DNA-binding activity of DAX-1

Zazopoulos et al. [16] have found that DAX-1 efficiently recognizes DNA hairpin structures in vitro. Binding is equally efficient to stems composed of 10–24 nucleotides, but is less efficient with shorter stems. The sequence of the loop influences DAX-1 binding: an adenine-rich sequence induces reduced binding compared with loops rich in cytosine or thymine. Methylation interference and distamycin-binding experiments indicate that DAX-1 predominantly interacts with the minor groove of the DNA helix. Zazopoulos et al. [16] have noted that these DNA-binding properties are reminiscent of high mobility group (HMG)-box proteins, such as SRY. However, whereas HMG proteins can bind to four-way DNA junctions, DAX-1 is unable to do so if the loop is missing. It is predicted that this new DNA-binding activity is a feature of the DAX-1 putative DBD. This domain has also been involved in protein-protein interaction (see below).

Does DAX-1 regulate expression of the *StAR* gene?

The spatial and temporal pattern of *DAX-1* expression in the adrenal cortex and Leydig cells suggested that it could be involved in the regulation of steroidogenesis. The steroidogenic acute regulatory protein (StAR) has a central role in steroidogenesis. Zazopoulos et al. [16] identified a DNA hairpin structure in the promoter of the *StAR* gene and demonstrated that it binds DAX-1. Transcription of the human *StAR* gene is induced by cyclic AMP (cAMP). DAX-1 represses both the basal expression and cAMP-induced activity of a *StAR* promoter reporter construct in the Y-1 cell line. A *StAR* promoter mutated in the hairpin structure is still cAMP-inducible, but is not repressed by DAX-1. In addition, *StAR* transcripts and protein are undetectable even after forskolin stimulation in Y-1 clones expressing DAX-1 after transfection [16]. Experiments using a hydroxylated cholesterol derivative show that biochemical steps in steroidogenesis subsequent to cholesterol delivery to mitochondria are also impaired in Y-1 cells expressing DAX-1. This is explained by the repression of P450scc and 3β-HSD expression, in addition to StAR. DAX-1 expression in Y-1 cells results in the inhibition of the activity of the StAR, P450scc and 3β-HSD promoters [23]. This suggests a prominent role for DAX-1 in the control of steroidogenesis. However, examination of a variety of adrenocortical tumours failed to detect the expected negative correlation between *DAX-1* and *StAR* expression [24].

Interaction between DAX-1 and SF-1 at the protein level

Interaction between SF-1 and DAX-1 proteins was demonstrated by Ito et al. [25] in a transient transfection model using the JEG-3 cell line. Transfection of an *SF-1* expression construct activates a reporter gene containing one or two copies of the SF-1 binding site. Cotransfection with a *DAX-1* expression construct reduces expression of the reporter gene. However, DAX-1 protein does not bind directly to the SF-1 site in gel-shift assays, nor does it alter the binding of SF-1 to its response element. In addition, cotransfection of *DAX-1* and *SF-1* did not apparently result in heterodimer formation; however, protein-protein interaction was detected in in vitro protein binding assays. DAX-1 C-terminal deletions (between amino acids 470 to 488) or the two naturally occurring amino acid substitutions R267P and DV269 failed to reduce DAX-1 binding to SF-1, whereas removal of the N-terminal region (amino acids 1 to 226) decreased binding.

A direct in vitro interaction between DAX-1 and SF-1 in the absence of DNA was confirmed (but not published) by Zazopoulos et al. [16], who were unable to demonstrate interaction in vivo. They proposed that repression by DAX-1 in a natural promoter context might require DNA binding.

More recently, Nachtigal et al. [26] showed that SF-1 acts synergistically with WT1 to promote anti-Müllerian hormone (*MIS*) expression (see Parker et al., this issue). This activation could be blocked by DAX-1. Removing both DAX-1 silencing domains (see below) was required to relieve the repression. Cotransfection of *DAX-1* and *SF-1* in the absence of WT1 did not cause a similar inhibitory effect. In a yeast two-hybrid assay, a prominent association between SF-1 and DAX-1, but not WT1 and DAX-1, was detected. Furthermore, binding between DAX-1 and SF-1 was also detected in a GST pulldown experiment.

Removal of the SF-1 LBD abrogated its interaction with GST-DAX-1, but not with GST-WT1, suggesting

that the major sites conferring interaction with DAX-1 and WT1 are mediated by different regions of SF-1. Nachtigal et al. [26] suggested that failure by other authors to detect a direct interaction in vivo might be due to the use of human DAX-1 versus mouse Sf-1, possibly due to the high degree of protein divergence between human and mouse DAX-1. Nachtigal et al. [26] also confirmed that DAX-1 is unable to bind or form a visible protein-protein complex on a SF-1 responsive element (MIS-RE-1).

The transcriptional silencing domains of DAX-1

The domains essential for transcriptional silencing were identified by the creation of a series of deletions and mutations within the C-terminus of DAX-1. These were fused in frame to the yeast GAL4 DBD [17, 25]. The effect of these fusion proteins on transcription driven by different promoters led to the identification of two silencing domains: removal of the most C-terminal 19 [16, 17] or 28 [25] amino acids results in almost complete abrogation of silencing. Lalli et al. [17] also examined the N-terminal portion of DAX-1 and demonstrated that deletions of amino acids 207–244 significantly reduces silencing in one of the systems used (fig. 1).

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