Natural Trans-spliced mRNAs Are Generated from the Human Estrogen Receptor-α (hERα) Gene*

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The human estrogen receptor-α (hERα) gene is a complex genomic unit exhibiting alternative splicing and promoter usage in a tissue-specific manner. During the investigation of new hERα mRNA variants by rapid amplification of 5′ cDNA ends, we identified a cDNA in which the acceptor site of exon 1A, into which the different leader exons are normally alternatively spliced, was spliced accurately the 3′ extremity of exon 1A (scrambled 1A→1A hERα cDNA). Reverse transcription-PCR and S1 nuclease mapping analysis revealed that 1A→1A hERα transcripts were not circular RNAs constituted by exon 1A only but corresponded to linear polyadenylated hERα RNAs composed of the eight coding exons of the hERα gene and characterized by a duplication of exon 1A. Genomic Southern blot experiments excluded the hypothesis of duplication of hERα exon 1A in the human genome. Therefore, these data suggested that 1A→1A hERα transcripts were likely generated by trans-splicing. The production of such transcripts by trans-splicing of pre-mRNAs generated from a chimeric gene formed by a single hERα exon 1A, exon 2, and their flanking intronic regions was demonstrated in transient transfection experiments. Therefore, in addition to the alternative cis-splicing, the hERα gene is also subject to natural trans-splicing.

The estrogen receptor-α (ERα) is a ligand-inducible transcription factor that belongs to the steroid, thyroid hormone, and retinoic acid receptor family (1–3). As all members of this family, it modulates transcription of specific sets of genes by interacting either in a protein/DNA manner with cognate DNA sequences called responsive elements or in a protein/protein manner with other transcriptional factors (1–5).

ERα is a key component of a wide range of biological processes. Its main role is in the control of the reproductive functions such as the establishment and maintenance of female sex differentiation characteristics, reproductive cycle, and pregnancy (6, 7). ERα is also involved in liver, fat, and bone cell metabolism, cardiovascular and neuronal activity, and embryonic and fetal development (6, 7). Finally, due to the mitogenic effect of its ligand, ERα is intimately associated with the biology of endometrium and breast cancers (8–10).

ER status is used clinically both as a prognostic factor and as a target in the therapy of breast cancers (9). Patients with ER-positive tumors have a better prognosis than those with tumors that lack ER expression. The benefits of the anti-estrogen therapy are almost limited to these patients, although quite a number of ER-positive tumors do not respond to endocrine therapy (8, 9). The resistance to hormonal therapy has often been associated with genetic defects within ER biology (11, 12). Thus, the identification of the molecular mechanisms controlling ERα expression and function and those that may impair ERα biology turned out to be a crucial step for understanding the involvement of the estrogen receptor into several physiological and pathological processes.

Mapped to the long arm of chromosome 6 (13), the human ERα gene is over 140 kb in length with a coding region split into eight exons (14). Our laboratory has recently shown that this gene is in fact a complex genomic unit exhibiting alternative splicing and promoter usage in a tissue-specific manner (15, 16). Using the rapid amplification of cDNA ends (RACE) methodology, we have isolated and characterized several new hERα cDNA isoforms and demonstrated that the hERα transcripts are produced from a single gene by the use of multiple promoters (16). Most of these hERα transcripts (A–F) encode a common ERα protein, hERα 66, but differ in their 5′ untranslated region as a consequence of an alternative splicing of several upstream exons (1B–1F) to a common acceptor site located in exon 1A, 5′ to the initiation of translation codon. A new class of hERα transcripts that lack the first coding exon (exon 1A) of the ERα gene was also identified (17). These Δ1A hERα transcripts originate from the E and F hERα promoters and encode the new N-terminal 173-amino acid truncated hERα 46 isoform (17).

During the RACE investigation, we amplified a hERα cDNA fragment in which the 3′ extremity of exon 1A was spliced directly to the acceptor site of the same exon 1A that normally receives the alternative upstream exons 1B–1F. In this present study, we demonstrate that this RACE product was not an artifact but rather results from the amplification of a hERα cDNA with a duplication of exon 1A. The new hERα transcripts correspond to trans-spliced mRNA.

EXPERIMENTAL PROCEDURES

Cell Lines and Tissues—The MCF7, T47D, ZR75–1, HEPG2, HOS TE67, SAOS, and HeLa cell lines were maintained in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen), penicillin (100 units/ml), and streptomycin (100 μg/ml) at 37 °C in a 5% CO2 incubator.

RNA Isolation—Total RNA from cell lines and tissues was extracted with TRIZol (Invitrogen) as described by the manufacturer. Total RNA
from human mammary gland, human endometrium, human brain, human liver, and human skeletal muscle were purchased from CLONTECH. Human pituitary RNA was kindly provided by Professor J. Duval (Université de Rennes, Rennes, France).

**Plasmid Construction**—The PCR-hERα Luc-plasmid was constructed as follows. DNA from the region of the luciferase gene was ampliﬁed from the pGL2 vector (Promega) using ﬂanking primers with BamHI restriction sites and was then inserted in the BamHI site of pCR 3.1 (Invitrogen) to obtain the pCR 3.1 Luc plasmid. The genomic fragments, a, b, and c (see Fig. 6), were ampliﬁed from the human GHER 1 and 3 clones in Bluescript (14) using the following primers: XhoI-a5′-5′-AGCTCTCGAGAAGACGGCCACGCTAC-3′ and M13 primer for fragment b; and M13 primer and KpnI-c3′-5′-AGCTGGTACACGAGGTCGCTGGTGTCAC-3′ and M13 primer for fragment a; and XhoI-b5′-5′-AGCTCGAGGAAGACGGCCACGCTAC-3′ and M13 primer for fragment c. A fragment a was digested by XhoI and KpnI and subcloned into the XhoI/XhoI site of pST 1 blue vector to form pST 1 blue vector a. Fragment b was digested by XhoI and EcoRI (site contained in Bluescript sequences) and subcloned into the EcoRI/KpnI site of pST 1 blue vector b. A fragment c was digested by EcoRI and KpnI and subcloned into the EcoRI/KpnI site of pST 1 blue vector c to form pST 1 blue vector a+b+c. Finally, pST 1 blue vector a+b+c was digested by NheI and KpnI, and the fragment NheI-a+b+c-KpnI was then inserted in the NheI/KpnI site of the pCR 3.1 Luc plasmid to form the PCR-hERα Luc plasmid.

**RACE**—The trans-spliced hERα mRNA (1A→1A) was cloned by an inverse PCR method (18). Reverse transcription of MCF7 total RNA (10 μg) and second-strand synthesis were performed using a commercial kit (Invitrogen) as recommended by the manufacturer except that the hERα gene-speciﬁc primer IV (5′-CTCACAAGGAGCACTGATCATGTT-3′) located in exon 2 was used instead of the usual oligo(dT) primer (see Fig. 1). Subsequently, the cDNA was circularized in the absence of T4 DNA ligase and subcloned to 35 rounds of PCR ampliﬁcation using the sense primer X (5′-ACTCAACAGCGTGTCTCCGAG-3′) and the antisense primer VI (5′-TTGGATCTGATGTCAGCATGGG-3′) (see Fig. 1). The main PCR product was subcloned in the TA cloning vector pCR 2.1 (Invitrogen) and then sequenced by the dye chain extension method.

**RT-PCR Analysis**—cDNAs were synthesized from 1 μg of total RNA following reverse transcription with 50 units of Expand™ reverse transcriptase (Roche Molecular Biochemicals) under the conditions recommended by the supplier using the oligonucleotide primer V (5′-TCTGACCGTGAAGTGTCGAG-3′) from hERα exon 1A (see Figs. 2B and 5A), primer IV from hERα exon 2 (see Figs. 1 and 6B), and primer I (5′-TCTGACGCTTCTAGTGGAGGGG-3′) from the 3′-untranslated region of hERα mRNA (exons 8) (see Fig. 5B), or primer L1 (5′-TCTAGAGGATAAGTGGCGC-3′) from the luciferase coding region in PCR-hERα Luc plasmid (see Fig. 6B). 2.5 μl of the reverse transcriptase reactions resulting from primer V were used as templates in either a 30-cycle PCR ampliﬁcation using primer X and primer VI (see Fig. 2) or in two round RT-PCR ampliﬁcation using the 5′-primer VIII (5′-GCCGCTTTGAGCTCCCTGCT-3′) and nested primer IX (5′-ATGGACGATCATGATCTCAAAACG-3′) with the 3′ primer VI and nested primer VII (5′-AAGGCTCAGAAGACGGCGGGG-3′) (see Fig. 5A). cDNAs reverse-transcribed from primer IV were ampliﬁed by PCR (30 cycles) using either the 5′ primer X and the 3′ primer VI (see Fig. 1) or the 5′ primer XI (5′-TCTGATAGCAGCCGACAGCGG-3′) and the 3′ primer VI (see Fig. 6B).

2.5 μl of the reverse transcriptase reactions resulting from primer I were used in two rounds of 30-cycle PCR ampliﬁcation (see Fig. 5B). The 5′ primer and nested primer used were X and VIII, respectively. The 3′ primer II (5′-ATTATTGACAGGTCGGTGAGG-3′) and the nested primer III (5′-CTGGAATGACAGCATGGTCTCAT-3′) were from the 3′-untranslated region of hERα cDNAs (exon 8). Both rounds of ampliﬁcation were performed using the Expand™ long template PCR system (Roche Molecular Biochemicals) as recommended by the manufacturer.

Finally, single-stranded cDNAs reverse-transcribed from primer L1 were subjected to either a 30-cycle PCR ampliﬁcation using the 5′ primer VIII and the 3′ primer L2 (5′-CCGGCTCTCTTATTTTTGTTTT-3′) (see Fig. 6A) or two rounds of 30-cycle PCR ampliﬁcation using the 5′ primer X and nested primer XI with the 5′ primer L2 and nested primer VI (see Fig. 6B).

5 μl from each reaction were analyzed on 1% agarose gels and transferred to nylon membranes (Hybond N+) by Southern blotting and hybridized with the random primed 32P-labeled probe 1A, as recommended by the manufacturer. Probe 1A is a genomic fragment from exon 1A (171 to +610 (21)) obtained by PCR ampliﬁcation.

**RESULTS**

**Evidence for the Existence of 1A→1A hERα Transcripts with the Donor Site of Exon 1A Joined to the Acceptor Site of Exon 1A**—To amplify new 5′ mRNA extremities of the hERα gene, a 5′ RACE approach based on a variation of the inverse PCR technique was performed on MCF7 hERα cDNA synthesized from primer IV located in exon 2 (Fig. 1A). Sequence analysis of the main RACE product (282 bp) showed that it corresponded to scrambled 1A→1A hERα transcripts with the donor site of exon 1A joined to the acceptor site of exon 1A (Fig. 1B), i.e. the alternative upstream exons 1B→1F are normally spliced (16). It should be noted that the hERα cDNA circularization step in the 5′ RACE approach was not required to amplify the 1A→1A hERα RACE product, which might explain its abundance. To conﬁrm the existence of such hERα transcripts, an S1 nuclease mapping experiment was performed on total RNA from various tissues or cell lines. The single-stranded DNA S1 probe was prepared from a 1A→1A hERα RT-PCR product as described under “Experimental Procedures.” This probe included 3′ end of hERα transcript 1A corresponding to 5′ exon 1A sequences and thus would not be completely protected if the standard transcripts were the only species present. After hybridizing probe 1A→1A with the RNA samples and S1 nuclease digestion, two protected fragments of 296 and 316 nucleotides were detected (Fig. 2A). As expected, the smallest fragment corresponded to normal A-F hERα mRNAs,
which remained homologous to probe 1A→1A as far as the acceptor splice site of exon 1A and then diverged in their 5' ends from probe complementary to 1A→1A sequences (16). The level and pattern of distribution of these hERα mRNAs were as described previously (16).
sequencing of the probe and therefore resulted from a hybridization with 1A→1A hERα transcripts (Fig. 2A). It was only weakly detected from MCF7 and T47D RNA samples. To study the tissue distribution of 1A→1A hERα transcripts by a more sensitive approach, an RT-PCR analysis was performed on the RNA samples reverse-transcribed from a hERα gene-specific primer (V) chosen in exon 1A (Fig. 2B). This study showed that 1A→1A hERα transcripts were detected by RT-PCR in tissues and cell lines expressing a relatively high level of normal hERα transcripts, for instance the mammary gland and the cell lines MCF7, T47D, and ZR75, which derive from this tissue, the endometrium, and the liver (Fig. 2B). It should be noted, however, that no amplification of 1A→1A hERα transcripts was obtained from ovary despite the detection of normal hERα transcripts by S1 nuclease mapping in this tissue.

1A→1A hERα Transcripts Likely Result from a Trans-splicing Reaction—To determine whether a hERα exon 1A duplication is present in the genome, human genomic DNA was digested with EcoRI and BamHI restriction enzymes and hybridized with an exon 1A probe (Fig. 3). The results of the Southern blot of both genomic digestions revealed a single hybridizing band, the size of which was in total agreement with the restriction enzyme map of the λ GHERα clones published previously (14). Therefore, exon 1A of the hERα gene was not duplicated.

Two other mechanisms might explain the detection of 1A→1A hERα transcripts: 1) the formation of circular 1A→1A hERα transcripts constituted by exon 1A only or 2) a trans-splicing reaction occurring naturally between two hERα pre-mRNAs (Fig. 4A). In this last case, 1A→1A hERα transcripts should contain additional exons of the hERα gene. To discriminate between these two hypotheses, an S1 nuclease mapping experiment was carried out using a probe designed to protect trans-spliced hERα transcripts with a 1A→1A-2 exon organization. Thus, if a trans-splicing reaction occurs for the hERα gene, then the corresponding protected fragment would be 624 nucleotides in size. On the other hand, the protection of a circular 1A→1A hERα transcript by probe 1A→1A-2 would give rise to a fragment of the size of exon 1A, 521 nucleotides. The S1 nuclease mapping analysis of MCF7 total RNA by probe 1A→1A-2 is shown in Fig. 4B. In addition to the 604-nucleotide fragment that results from a protection of probe 1A→1A-2 by normal hERα transcripts up to the acceptor splice site of exon 1A, the results also showed a protected fragment of 624 nucleotides in size, thus demonstrating the trans-splicing origin of the scrambled 1A→1A hERα transcripts. This result was strengthened by the detection of the 1A→1A-2 protected fragment in the RNA poly(A⁺) fraction, which indicated that 1A→1A hERα transcripts are polyadenylated molecules. Finally, no protected fragment corresponding in size to circular 1A→1A hERα transcripts was seen in this S1 nuclease mapping experiment.

Since 1A→1A hERα transcripts should contain the remaining exonic segments of the hERα gene that a trans-splicing process would be likely to generate, the exonic organization of 1A→1A hERα transcripts was investigated. Firstly, to verify that a full exon 1A was present in 5’ to the 1A→1A junction, hERα transcripts from various sources of RNA were reverse-transcribed from primer V in exon 1A, and two rounds of PCR were then performed to amplify a fragment of 1A→1A hERα cDNAs containing the anticipated sequences as illustrated in Fig. 5A. A PCR product of the expected size was amplified from the tissues or the cell lines in which the 1A→1A hERα transcript was detected previously by RT-PCR (Fig. 5B). The specificity of this product was further confirmed by Southern blot using the exon 1A-specific oligonucleotide probe P2. Secondly, to demonstrate that full-length 1A→1A hERα transcripts had hERα sequences from exon 1A through to exon 8 (3’ to the 1A→1A junction), PCR analysis was performed on single-strand cDNAs synthesized using a hERα gene-specific primer (I) chosen from the hERα mRNA 3’-untranslated region sequences (exon 8, Fig. 5B). 1A→1A hERα cDNAs were amplified by two rounds of PCR using the 3’ primer II and nested primer III located upstream from primer I in exon 8 in combination with the 5’ primer X and nested primer VIII (Fig. 5B). It should be noted that the first round of PCR amplified both 1A→1A and normal hERα cDNAs. Only the second round allowed to be specifically amplified 1A→1A hERα cDNAs. Results showed that the size of the amplified cDNAs was as expected, and after Southern blotting, the hybridization of these PCR products with various oligonucleotide probes recognizing specifically the different eight coding exons of the hERα gene demonstrated that sequences from exon 1A to exon 8 were present in 1A→1A hERα transcripts (Fig. 5B only shows the results obtained with the exon 1A-specific oligonucleotide probe P2). In conclusion, these data clearly demonstrated the existence of a new class of hERα mRNAs that presents a duplication of exon 1A and which is likely generated by a trans-splicing event between two hERα pre-mRNAs.

A Chimeric Gene Containing hERα Exon 1A, the 5’ Part of Exon 2, and Their Flanking Intronic Sequences Generate Trans-spliced 1A→1A Transcripts—To further define the mechanism generating 1A→1A hERα transcripts, a chimeric gene called pCR-hERα Luc was constructed and analyzed for its ability to generate 1A→1A trans-spliced transcripts after transient expression in the MCF7 cell line. pCR-hERα Luc was formed by the cytomegalovirus promoter, the hERα genomic region from exon 1B to an EcoRI restriction site in the 3’-flanking intronic region of exon 1A, a part of hERα exon 2 and its 5’-flanking sequence to an EcoRI restriction site, the luciferase coding region, and the 3’-untranslated region of the bo-
vine growth hormone (see “Experimental Procedures” for the construction of pCR-hERα Luc) (Fig. 6A). To discriminate 1A−→1A hERα cDNAs generated from the chimeric gene from those arising from the standard hERα gene expression in MCF7 cells, an XhoI restriction site was created in the 3′ extremity of exon 1A of pCR-hERα Luc gene. Thus, total RNA prepared from MCF7 transiently transfected with pCR-hERα Luc was used to reverse-transcribe hERα Luc mRNA from primer L1 located in the 5′ end of the luciferase coding region. As illustrated in Fig. 6A, hERα Luc mRNA was accurately matured since the size of the hERα Luc cDNA PCR-amplified between exon 1A and the luciferase coding region indicated that exon 1A was spliced as expected to exon 2 with the removal of the ~2.5-kb intronic region. Then, in attempt to amplify trans-spliced 1A−→1A hERα Luc cDNAs, two rounds of PCR were performed on hERα Luc cDNAs reverse-transcribed from primer L1 as described in Fig. 6B. The result showed one main PCR product, the size of which was in agreement with the one expected from the amplification of 1A−→1A hERα transcripts but was also able to partially protect the A/F hERα mRNA isoforms (Σ→1A−→1A hERα transcripts) up to the splice site position. The probe was designed to contain vector sequence in its extremity (denoted by the thinner black line) to discriminate between undigested probes (<> and specific protected fragments. Positions of migration of the molecular size markers are shown on the left side of the figure.

Fig. 4. 1A−→1A hERα transcripts are linear polyadenylated molecules. A, schematic diagram of the two hypotheses proposed to explain the detection of 1A−→1A hERα transcripts: 1) circular RNAs constituted by exon 1A only or 2) linear RNAs formed by a trans-splicing reaction occurring naturally between two hERα pre-mRNAs. B, S1 nuclease mapping experiment was carried out to discriminate between these two hypotheses. It was performed as described under “Experimental Procedures” with the single-stranded probe 1A−→1A-2, designed to protect linear trans-spliced hERα transcripts with a 1A−→1A-2 exon organization, and 30 μg of MCF7 total RNA, 30 μg of MCF7 poly(A)+ RNA, or 0.1 μg of MCF7 poly(A)+ RNA mixed with 30 μg of yeast total RNA. Yeast total RNA (30 μg) was used as a negative control. The location and the size of the single-stranded probe 1A−→1A-2 and the protected fragments obtained after S1 digestion are indicated. The probe was specific for 1A−→1A hERα transcripts but was also able to partially protect the A/F hERα mRNA isoforms (Σ→1A−→1A hERα transcripts) up to the splice site position. The probe was designed to contain vector sequence in its extremity (denoted by the thinner black line) to discriminate between undigested probes (<> and specific protected fragments. Positions of migration of the molecular size markers are shown on the left side of the figure.
formed by hERα exon 1A, exon 2, and their flanking intronic regions is able to generate trans-spliced 1A→1A hERα transcripts and therefore contains all information required for this process.

**DISCUSSION**

In this investigation, we have demonstrated that the human estrogen receptor-α gene is able to generate novel hERα mRNAs by trans-splicing. The new class of hERα mRNAs presents a duplication of exon 1A and is referred to as trans-spliced 1A→1A hERα transcripts.

Heterogeneity in the 5’ ends of mRNAs generated by alternative promoter usage and splicing is a common feature among the members of the steroid/thyroid hormone/retinoic acid receptor gene family (22–25). For the human, mouse, rat, or chicken ERα genes, several 5’ end variants of ERα mRNAs produced by the splicing of alternative untranslated upstream exons to the first translated exon were reported (16, 26–28). Most of these ERα mRNA variants were identified by 5’ RACE. Surprisingly, when applied to human ERα mRNAs, this approach allowed us to amplify a new type of hERα cDNA. This variant, in contrast to the other amplified hERα cDNAs (A-F hERα cDNAs (16)), presented the 3’ part of exon 1A in 5’ to the acceptor of exon 1A, where normally the alternative upstream exons 1B–1F are spliced. The scrambled 1A→1A hERα cDNA showed an accurate junction between the donor site of exon 1A and the acceptor site of exon 1A, which might indicate that it arises from a natural phenomena rather than a RACE artifact.

The existence of the 1A→1A hERα transcripts in estrogen target cells was further confirmed by RT-PCR and S1 nuclease mapping experiments. In contrast to a previous report on a genomic rearrangement in an estrogen-independent subclone of the MCF7 human breast cancer cell line in which hERα exons (exons 6 and 7) were duplicated in an in-frame fashion (29), the hypothesis of a duplication of hERα exon 1A in the human genome was ruled out after a genomic Southern blot experiment. Results always revealed a single hybridizing band, demonstrating that this segment of the hERα gene is not duplicated. Furthermore, 1A→1A hERα transcripts were detected in several healthy tissues, excluding a genomic rearrangement origin associated with a pathological process.

Exon scrambling is an event that has often been also associated with circular RNA molecules (30, 31). Such RNAs were described for the testis-determining gene Sry (32). For the human _ets-1_ gene (33), the human cytochrome p-450 2C18 gene in epidermis, and the rat androgen-binding protein gene in testis (31). Exons skipped during alternative pre-mRNA processing could be indeed present in a circular molecule that has the donor site of the 3’ exon joined to the acceptor site of the 5’ exon. Accordingly, if 1A→1A hERα transcripts are the result of such a process, it would be expected that they are circular molecules composed of one single exon, exon 1A, joined at the 5’ and 3’ splice junctions, as in the case of the circular sry transcript in adult mouse testis (32). However, the presence in the transcripts of the other coding exons
of the hERα gene as well as the fact that they are polyadenylated molecules clearly demonstrates that hERα transcripts are not circular RNAs but rather linear molecules that probably result from a trans-splicing event between two hERα pre-mRNAs.

Trans-splicing is a post-transcriptional process occurring during the mRNA maturation in which RNA segments of two independent transcripts are spliced together to generate a new mRNA species. This mechanism was first demonstrated in trypanosomes (34) and subsequently reported in nematodes (35), flatworms (36), and plant cell organelles (37). In mammalian cells, trans-splicing events were suggested by computer analysis (38) and then by in vitro and in vivo experiments (39–42). Mammalian cell extracts have been demonstrated to have the ability to join RNA segments together by trans-splicing (39). More recently, trans-splicing reactions between synthetic pre-mRNA substrates were shown in in vitro studies and require either a downstream 5′ splice site or exonic enhancers (40, 41). Finally, SV40 transcripts trans-spliced to each other were detected in cells transformed by an early SV40 DNA fragment (42). Naturally occurring pre-mRNA trans-splicing in mammalian cells has not been frequently reported. The first indications of its existence were based on cDNA sequencing experiments, but alternative cis-splicing could not be excluded. Recently, additional reports strengthened the idea that trans-splicing events occur in mammalian cells and contribute to mRNA generation. In rat liver cells, Caudevilla et al. (43) have identified carnitine octanoyltransferase mRNA variants with a duplication of exon 2 or exons 2 and 3, which is not found in genomic DNA. Splicing experiments carried out in vitro with exon 2, which is specific for exon 1A, was then used in a first round of PCR amplification with primer L2, which is nested to primer L1. A second round of PCR reaction was performed with the primers XI and VI as illustrated on the schematic diagram. As a control, the endogenous trans-spliced 1A→IA hERα transcript was also reverse-transcribed from primer IV and PCR-amplified using the primer XI and VI. After purification, the PCR products were or were not digested with the restriction enzyme XhoI, electrophoresed through an agarose gel, and transferred by Southern blot to a membrane, which was then hybridized with the oligonucleotide probes P1 and P3 specific for the 5′ and 3′ regions of exon 1A, respectively.
study that natural trans-spliced 1A→1A mRNA is generated from the hERα gene, and such a process can be mimicked in vivo with a chimeric gene containing hERα exon 1A, the 5′ part of exon 2, and their flanking intronic sequences.

If 1A→1A hERα transcripts result from a trans-splicing event, it is likely that this process would generate two products: a long mRNA having a duplication of exon 1A (1A→1A hERα mRNA) and a short mRNA lacking exon 1A (Δ1A hERα mRNA). RT-PCR analysis clearly confirmed, in several ERα-positive tissues or cell lines, the presence of the long 1A→1A trans-spliced hERα mRNA composed by the eight coding exons of the hERα gene including a duplication of the first coding exon, exon 1A. Upstream to the main open reading frame that encodes hERα 66, 1A→1A hERα mRNA presents a second open reading frame, shared by the trans-spliced exon 1A and the 5′ part of the acceptor exon 1A, which would encode for a protein of 156 amino acid residues, equivalent to the A/B domain of hERα. Such a protein will be unable to bind directly DNA but would contain the transactivation domain AF1, which could constitutively act by interacting with coactivators such as SRC-1 or the p68 and p72 RNA helicases (45, 46) and as a constitutive act by interacting with coactivators such as SRC-1 or the p68 and p72 RNA helicases (45, 46) and as a

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Natural Trans-spliced mRNAs Are Generated from the Human Estrogen Receptor-α (hER α) Gene
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