ANTIOXIDANTS & REDOX SIGNALING
Volume 9, Number 8, 2007

DOI: 10.1089/ars.2007.1550

Comprehensive Invited Review

Nucleoredoxin, a Novel Thioredoxin Family Member Involved in Cell Growth and Differentiation

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Reviewing Editors: Arne Holmgren, Hajime Nakamura, Lloyd Ruddock and Junichi Sadoshima

ABSTRACT

Thioredoxin (TRX) family proteins are involved in various biologic processes by regulating the response to oxidative stress. Nucleoredoxin (NRX), a relatively uncharacterized member of the TRX family protein, has

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recently been reported to regulate the Wnt/β-catenin pathway, which itself regulates cell fate and early development, in a redox-dependent manner. In this review, we describe the TRX family proteins and discuss in detail the similarities and differences between NRX and other TRX family proteins. Although NRX possesses a conserved TRX domain and a catalytic motif for oxidoreductase activity, its sequence homology to TRX is not as high as that of the close relatives of TRX. The sequence of NRX is more similar to that of tryparedoxin (TryX), a TRX family member originally identified in parasite trypanosomes. We also discuss the reported properties and potential physiologic roles of NRX. *Antioxid. Redox Signal.* 9, 1035–1057.

I. INTRODUCTION

Reactive oxygen species (ROS) are oxygen-containing molecules that possess a stronger reactivity than oxygen itself. ROS include the superoxide radical ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($^{\cdot}OH$), among others. ROS are inevitable byproducts of normal cell activities, and because they are highly reactive, they can modify various cellular molecules (proteins, lipids, and nucleic acids). Therefore, excessive ROS production is known to be harmful to cells. Cells respond to ROS by activating stress signaling pathways and limit ROS-induced damage by enhancing antioxidant systems, which act to detoxify ROS. When the ROS challenge is extremely strong, it can alter cell function and lead to cell death. However, cells also generate ROS themselves to use them as mediators of intracellular signaling pathways (34, 168). Good examples are the signaling pathways stimulated by platelet-derived growth factor (PDGF) and other growth factors (171). These reports emphasize the importance of redox-signaling research to understanding the intracellular signaling network.

Many molecules are involved in the antioxidant system. Superoxide dismutase (SOD) can metabolize $O_2^{\cdot -}$. Catalase directly quenches H_2O_2. Thioredoxin (TRX) is a thiol oxidoreductase that is essential for DNA synthesis through ribonucleotide reductase and for various redox signaling pathways (56). TRX can scavenge ROS mainly by reducing various target proteins, including the H_2O_2-quenching enzyme peroxiredoxin [Prx; six human Prx members are known (144)]. TRX also regulates stress signaling pathways (37). TRX and related proteins compose a large family. However, the functions of TRX family members, with the exception of TRX1, are not well understood.

In this review, we focus on the roles of the various TRX family members, with special emphasis on nucleoredoxin (NRX), a relatively uncharacterized TRX family member (88). Recent reports from our group and others have shed light on the physiologic functions of NRX. NRX shows significant sequence homology to TRX, but it is more homologous to tryparedoxin (TryX), a TRX family protein first identified in parasite trypanosomes (158). To allow comparison between NRX and other TRX family molecules, we first briefly summarize what is known regarding the major members of the TRX family and then discuss NRX in detail. Our recent study showed that NRX is a redox sensor that acts to regulate the signaling of the Wnt/β-catenin pathway (39), a well-characterized pathway involved in cell proliferation and differentiation (20, 120, 125, 143). We also discuss other reports on NRX, including its identification, expression pattern, and binding proteins and the potential of NRX research in biology and medicine.

II. THIOREDOXIN (TRX) AS AN OXIDOREDUCTASE

TRX was first identified as an electron donor for ribonucleotide reductase for DNA synthesis in *Escherichia coli* (92). TRX is a small protein with a molecular mass of ~12 kDa (human TRX possesses 105 amino acids), and it is conserved throughout species in prokaryotes and eukaryotes (29). Human TRX1 was first identified in platelets and fibroblasts (9, 90, 109). TRX also is referred to as adult T-cell leukemia–derived factor (ADF), which is secreted from adult T-cell leukemia cell lines and induces the expression of the interleukin 2 receptor (175), and as "early pregnancy factor" (19), a very early serum marker for fertilization. TRX possesses a conserved WCGPC (Trp-Cys-Gly-Pro-Cys) motif, and the two cysteine residues (Cys32 and Cys35 in human TRX1) are directly involved in oxidoreductase reactivity (59). Many proteins are reported as substrates for the oxidoreductase activity of TRX, including peroxiredoxin (Prx) (144), ribonucleotide reductase (58), and methionine sulfoxide reductase (165). Prx is known to neutralize H_2O_2, and this activity is efficiently reconstituted *in vitro* with TRX, thioredoxin reductase (TrxR), and NADPH (Fig. 1a).

With respect to the mechanism of the oxidoreductase activity of TRX, it is known that the two Cys residues of the WCGPC catalytic motif play different roles (Fig. 2) (77). The thiol group of the N-terminal Cys residue (Cys32 in human TRX1) attacks one of the sulfur atoms of the Cys residues that form disulfide bonds in substrate proteins. The thiol group of the N-terminal Cys residue is easily deprotonated by its surrounding conditions and residues, including Asp26 in human TRX1, and it can therefore easily execute a nucleophilic attack (26). This results in the formation of a reaction intermediate between TRX and the substrate protein that is linked with a disulfide bond. The subsequent reaction is performed by the thiol group of the C-terminal Cys residue (Cys35 in human TRX1), which attacks the sulfur atom of the N-terminal Cys residue forming the disulfide bond in the intermediate complex and releases the substrate protein from TRX. A disulfide bond is now formed between the two reactive Cys residues of TRX itself. This disulfide bond of TRX is reduced by TrxR (180). Mammalian TrxR is a se-

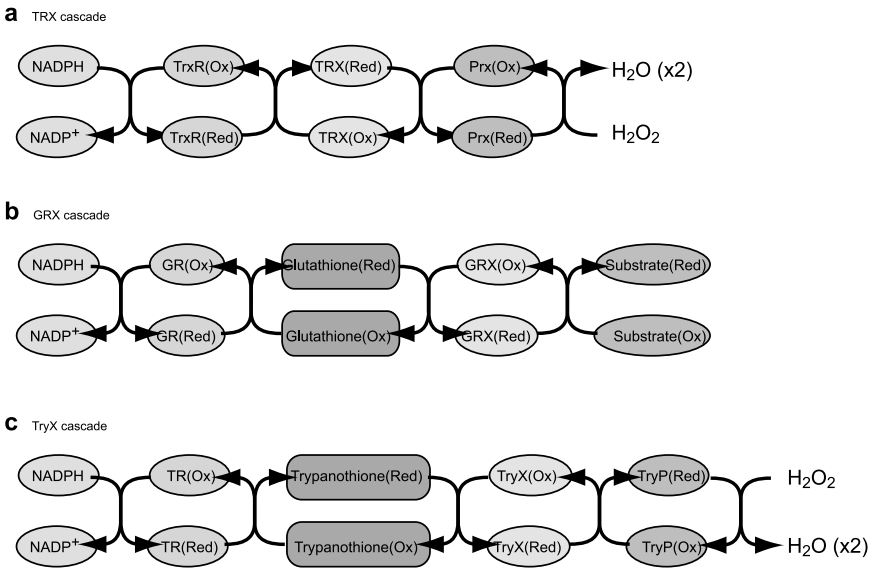

FIG. 1. Redox cascades. (**a**) Thioredoxin (TRX) cascade. TRX is an oxidoreductase that can reduce the disulfide bonds of various target proteins, the most important with respect to reactive oxygen species (ROS) scavenging being peroxiredoxin (Prx). TRX generates a TRX cascade, comprising NADPH, TRX reductase (TrxR), TRX, and Prx. NADPH serves as an electron donor for the entire cascade. It reduces TrxR, and TrxR then reduces TRX. Reduced TRX reduces Prx, which effectively quenches ROS. (**b**), Glutaredoxin (GRX) cascade. The function of this cascade is similar to that of the TRX cascade (*i.e.*, to relay an electron from NADPH to the substrate protein). The major difference is the intermediate glutathione between glutathione reductase (GR) and GRX. (**c**) Tryparedoxin (TryX) cascade. TR, trypanothione reductase; TryP, TryX peroxidase. The trypanosomiasis redox cascade resembles that of the GRX system (**b**), but it uses trypanothione instead of glutathione.

lenocystein-containing protein, and it uses NADPH to reduce itself (40, 177, 209). On the whole, the TRX cascade delivers the redox potential from NADPH to the substrate protein *via* TrxR and TRX (see Fig. 1a).

III. TRX FAMILY PROTEINS

Many mammalian proteins that possess a TRX-related domain are represented in databases (we refer mainly to the NCBI database. The accession numbers are provided in the figure legends) and compose the TRX family (63, 104). This large family can be divided into subfamilies based on several criteria (Fig. 3). It should be noted that Prx also contains an expanded TRX-like domain (195), and along with other proteins (such as glutathione peroxidases), forms an even larger TRX superfamily. We briefly discuss some of the TRX family members. Original reports and detailed reviews can be found in the literature (63, 104, 122).

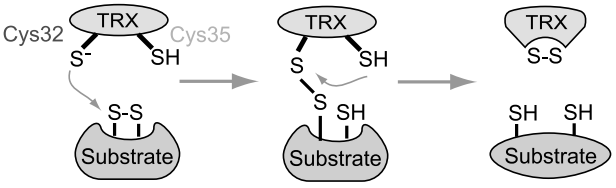

FIG. 2. Mechanism of disulfide bond reduction by TRX. Two conserved cysteine residues are found in the catalytic center of TRX (Cys32 and Cys35). The reaction is initiated with a nucleophilic attack by the Cys32 residue to one of the Cys residues composing the disulfide bond of the substrate protein. This results in the generation of an intermediate reaction in which TRX and the substrate protein are linked by the disulfide bond. Cys35 then attacks the Cys32 residue. The reaction is terminated by the generation of disulfide bond–containing (*i.e.*, oxidized) TRX and reduced substrate protein.

A. TRX1

Two mammalian TRX proteins are known, TRX1 and TRX2 (164). TRX1 is the best-characterized protein in the TRX family. This ubiquitous protein has multiple functions. It plays an important role as a key molecule in a ROS-neutralization cascade composed of NADPH, TrxR, TRX, and Prx. In this cascade, TRX1 reduces Prx, which then directly and efficiently neutralizes ROS to reduce peroxides. Several reports indicate that TRX1 also is able to neutralize H_2O_2 directly and that it can also protect cells from oxidative stress by reducing incorrectly formed disulfide bonds of various cellular proteins. TRX1 therefore protects cells from oxidative stress *via* its oxidoreductase activity. In addition to Prx, TRX1 has many other specific substrate proteins (Table 1). (TRX can also reduce the disulfide bonds of many other proteins that are generated under oxidative stress.) TRX1 serves as an electron donor for many enzymes, such as ribonucleotide reductase and methionine sulfoxide reductase (58, 165). It is known that TRX1 localizes mainly in the cytosol and that it moves to the nucleus when cells are stimulated by various ROS-generating treatments [*e.g.*, H_2O_2 itself, tumor necrosis factor-α (TNF-α), and UV]. In the nucleus, TRX modulates the redox status of transcription factors and their regulating proteins [*e.g.*, nuclear factor-κB (NF-κB) and redox factor-1 (Ref-1)] and controls transcriptional activity (46, 52, 156).

In addition to its oxidoreductase activity, TRX1 has been reported to bind to apoptosis signal-regulating kinase 1 (ASK1), a mitogen-activated protein kinase kinase kinase (MAPKKK) involved in apoptosis, in a redox-dependent manner (Fig. 4)

(68, 155). Various cellular stresses and inflammatory cytokines cause ROS production and induce the oxidization of TRX1. Oxidized TRX1 dissociates from ASK1. Free ASK1 then self-activates and activates the downstream stress kinase pathway. ASK1 directly phosphorylates its substrate MAPKKs, MKK4/MKK7, and MKK3/MKK6. MKK4/MKK7 activates c-Jun N-terminal kinase (JNK), and MKK3/MKK6 activates p38 MAPK. Both JNK and p38 MAPK induce apoptosis. This signal cascade controlled by the nonenzymatic function of TRX1 is important for the initiation of apoptosis when cells are exposed to strong oxidative stress that exceeds the detoxifying capacity of intracellular antioxidants.

TRX1 also functions extracellularly. As mentioned earlier, human TRX1 was also identified as adult T-cell leukemia–derived factor, an autocrine factor that induces the expression of the interleukin-2 receptor in human T-lymphotrophic virus-I–transformed cells (175). It is known that TRX1 and its truncated form [*i.e.*, TRX80 (135)] are secreted from cells when they are subjected to various stress conditions such as infection with human immunodeficiency virus or development of rheumatoid arthritis (112, 123, 202). Although the mechanism has not been fully elucidated, secreted TRX1 is reported to stimulate the proliferation and chemotactic movements of immune cells (7, 8, 124).

The physiologic importance of multifunctional TRX1 is reflected by the severe phenotype of *TRX1*-deficient mice. *TRX1*-null homozygotes are severely affected at the peri-implantation stage, and they die within 6.5 days postcoitum [dpc (110)]. An interesting report that was recently published by Conrad's group (71) showed that mice deficient in *TRXR1* (the gene encoding TrxR1, which selectively reduces TRX1, whereas TrxR2 reduces TRX2) die around embryonic day (E) 10.5. The difference in phenotypic severity between these two knockout strains may be due to functions of TRX1 apart from its oxidoreductase activity.

B. TRX2

In 1997, Spyrou *et al.* (164) reported a novel protein highly similar to TRX and named it TRX2. Interestingly, unlike TRX1, TRX2 localizes to the mitochondria. The tissue distribution of TRX2 also differs from that of TRX1. TRX1 is expressed ubiquitously, whereas TRX2 is expressed strongly in the heart, skeletal muscle, cerebellum, adrenal grand, and testis. TRX2 possesses oxidoreductase activity, and along with mitochondrial TrxR and Prx3, scavenges H_2O_2 and regulates the redox status. It also binds to mitochondrial ASK1 and prevents ASK1-induced apoptosis (see Fig. 4) (206). A *TRX2*-deficient cell line (178) shows massive apoptosis, and a similar tendency is observed in *TRX2*-deficient mice, which die, probably because of an inability to maintain mitochondrial redox homeostasis and abnormal activation of the ASK1 apoptosis pathway (129).

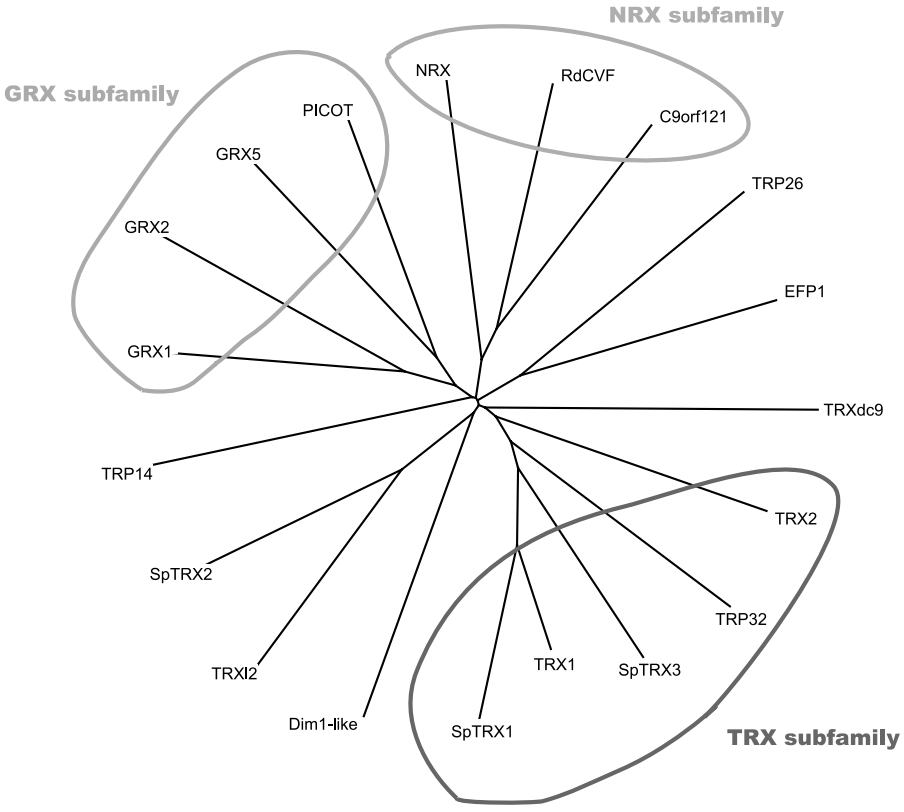

FIG. 3. TRX family members. Dendrogram of TRX family proteins (generated by Clustal W http://www.ebi.ac.uk/clustalw/. PDI and TMX proteins were omitted because so many proteins are included in this category, and their inclusion would make the dendrogram too complicated). In this sequence comparison, we can find known TRX and GRX subfamily proteins and equally well clustered third putative subfamily "NRX (TryX-like) subfamily." The accession numbers are as follows; TRX1: NP_003320, TRX2: AAH50610, GRX1: AAC35798, GRX2: CAI10820, GRX5: AAZ30731, PICOT (TRX-like 3): CAC40691, TRP14: NP_116120, TRP32: NP_004777, TRP26: NP_065095, TRX-like 2 (TRXl2): AAG28497, EFP1 (TRX domain containing 11): NP_056998, SpTRX1: NP_115619, SpTRX2: Q8N427, SpTRX3: NP_001003936, TRX domain containing 9 (TRXdc9): AAH70183, Dim1-like: NP_006692, NRX: NM_022463, RdCVF: AAH14127, C9orf121: Q5VZ03 (all *Homo sapiens*).

TABLE 1. KNOWN SUBSTRATES OF TRX

Substrates of TRX Protein	*Function*
Methionine sulfoxide reductase	Reduction of oxidized methionine
NF-kB	Modulation of transcriptional activity
Peroxiredoxin	ROS elimination
Ref-1	Modulation of transcriptional activity
Ribonucleotide reductase	DNA synthesis

C. Glutaredoxin (GRX)

GRX is another well-characterized member of the TRX family. It was first discovered in *Escherichia coli* by Holmgren (57), as a glutathione-dependent electron donor for ribonucleotide reductase. Mammalian GRX was subsequently purified from calf thymus (108, 109). Human GRX1 was purified and cloned from placenta (132). The most characteristic difference between GRX and TRX is that GRX uses glutathione as a direct hydrogen donor. The cascade includes NADPH, glutathione reductase, glutathione, and GRX (see Fig. 1b). In mammals, three GRX proteins are known: GRX1, 2, and 5 (no GRX3 or 4 occurs in mammals; these were first identified in *Saccharomyces cerevisiae*) (149). GRX1 and 2 possess TCPYC and SCYSC catalytic motifs, respectively, and both possess oxidoreductase activity (62). However, the catalytic motif in GRX5 is QCGFS; that is, no second Cys residue exists, which is essential for oxidoreductase reactivity in other TRX family members. However, another Cys residue has been reported in yeast GRX5 at the C terminus; this residue functions in a manner similar to the second Cys residue in the conservative CXXC motif (176). GRX5 is essential for viability in yeast and zebrafish, probably because of its role in the mitochondrial biogenesis of Fe-S clusters (150, 193). No reports regard mammalian GRX5 other than its database registration.

Similar to TRX1, GRX1 participates in various redox-related phenomena. It acts to reduce the disulfide bonds of many proteins, such as ribonucleotide reductase, to facilitate enzymatic reactions (60). GRX1 also regulates several transcription factors (53) and controls apoptosis (18). GRX1 has a distinct function as a reducing agent of protein–glutathione mixed disulfides [deglutathionylation (62, 161)] *via* its selectivity for the gamma-isopeptide bond found in glutathione (136).

Similar to GRX5, GRX2 appears to be involved in the biogenesis of Fe-S clusters (103). GRX2 is localized predominantly to the mitochondria (and partially to the nucleus), and GRX2-depleted cells induced by RNA interference (RNAi) show increased sensitivity to doxorubicin and phenylarsine oxide, which are ROS-inducing reagents, even though the cellular content of GRX2 is 20-fold less than that of GRX1 (105). These results suggest that GRX2 has a special role in the protection of cells from ROS, apart from that played by GRX1. How GRX2 and GRX5 cooperate with each other during the biogenesis of Fe-S clusters remains to be determined.

D. TRX-related protein 14 (TRP14)

TRP14 was identified as a protein with a ROS-sensitive Cys residue (75). The catalytic motif of TRP14 is WCPDC. TRP14 possesses TRX-like oxidoreductase activity and can quench H_2O_2 directly. However, it does not efficiently reduce several known TRX substrates such as Prx and insulin [insulin is not a physiologic substrate of TRX, but is widely used to examine the oxidoreductase activity of TRX domain–containing proteins experimentally (61)]. TRP14 appears to be expressed ubiquitously in tissues and cell lines and is localized to the cytosol.

TRP14 modulates TNF-α–induced NF-κB signaling and can suppress TNF-α–induced apoptosis. However, the mechanism appears to differ from that of TRX, and TRP14 does not bind

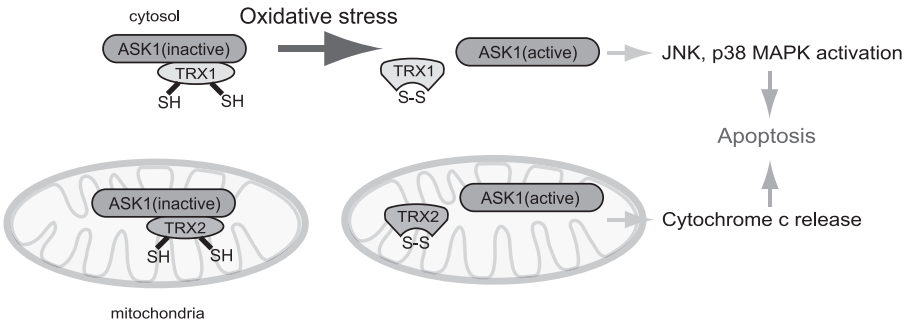

FIG. 4. TRX/ASK1 system. In the absence of cellular stressors, TRX is in the reduced form and can efficiently bind to ASK1 (TRX1 binds to the cytosolic ASK1, whereas mitochondrial TRX2 binds to the ASK1 in the mitochondria). When cells are challenged with stressors and the cellular ROS level increases, TRX becomes oxidized and changes its conformation. As a consequence, it dissociates from ASK1, which results in its self-activation. The upregulation of the stress kinase pathway by cytosolic Ask1 results in the activation of JNK and p38 MAPK. Together with the cytochrome *c* release from the mitochondria, which appears to be regulated by the ASK1/TRX2 complex, these events result in apoptosis.

ASK1 (74). LC8/PIN (51) has been identified as a candidate for a specific substrate of TRP14. LC8/PIN is a dynein light-chain protein that functions as an inhibitor of neuronal nitric oxide synthase (nNOS) (70). It also binds IκB and Bim (a Bcl-2 family member) (22, 141). It is speculated that TRP14 regulates NF-κB signaling and apoptosis by modulating the redox status of LC8/PIN and its binding to IκB or Bim.

E. TRX-related protein 32 (TRP32)

TRP32 was initially copurified with the catalytic fragment of a serine/threonine kinase mammalian STE-20-like (MST) (94). MST is a mammalian homologue of STE-20 like a molecule that possesses a kinase domain similar to that of STE-20 (mammalian p21-activated kinase; PAK). It was recently reported that MST phosphorylates forkhead transcription factor (FOXO)—a transcription factor involved in cell proliferation and longevity—when cells are stimulated by ROS. Phosphorylated FOXO translocates to the nuclei of neurons and induces cell death (96). They also reported that TRP32 and MST do not coimmunoprecipitate (96). Therefore, it appears that they do not exist as a stable intracellular protein complex. However, it is possible that TRP32 regulates the MST kinase activity induced by oxidative stress *via* the modulation of Cys residues; TRP32 possesses oxidoreductase activity.

F. Sperm-specific TRX (SpTRX)

To date, three SpTRX proteins, SpTRX1-3, have been reported (76, 118, 154). They are all identified as gene products that show sequence homology to TRX and are expressed exclusively in the testis. They possess a common TRX-like domain, but other domain structures differ.

SpTRX1 possesses 23 repeats of a 15-amino-acid sequence [QPKXGDIPKS(P/S)E(K/E)XI] in its N-terminal half. The function of this sequence stretch remains unknown. Wild-type SpTRX1 possesses significant catalytic activity for insulin reduction. However, a truncated mutant possessing only the TRX-like domain does not reduce insulin efficiently. The N-terminal portion of SpTRX1 can be modulated by phosphorylation through protein kinase C or by ubiquitination (118).

SpTRX2 has three nucleoside diphosphate (NDP)-kinase domains in its C-terminal half. However, neither NDP-kinase activity nor TRX-like oxidoreductase activity has been detected. One possibility is that the TRX-like domain and the NDP-kinase domain compete with each other. Significant oxidoreductase activity was observed in an insulin-reduction assay with the truncated form of SpTRX2 containing the TRX-like domain alone. SpTRX3 contains only the TRX domain. However, it does not show effective oxidoreductase activity. The authors argued that cofactors may be required for enzymatic activity.

G. Protein disulfide isomerase (PDI)

Protein disulfide isomerases (PDIs) comprise a large subfamily of TRX-like proteins that localize to the ER. PDIs facilitate proper protein folding and regulate oxidation and isomerization reactions in the ER (191). To date, 18 members of the human PDI family have been described (30), and TRX-related transmembrane proteins [TMX (45, 111, 114)] are also regarded as members of the PDI family because they localize to the ER. PDIs are numerous and have many functions, the description of which is beyond the focus of our review. For further details, please refer to other reviews focusing on PDI and other members in this family [for example, (30) and (78)].

IV. IDENTIFICATION OF THE NUCLEOREDOXIN (NRX) GENE

Nucleoredoxin (NRX, NXN, or Red-1) was first identified by Kurooka *et al.* (88). The *NRX* gene was discovered during a search for novel genes around the *nude* (also known as *Foxn1, Whn,* or *Hfh11*) gene locus. It was named nucleoredoxin because the sequence of its gene product is similar to that of the TRX protein, and NRX localized predominantly to the nucleus when it was ectopically expressed in COS-7 cells. However, the subcellular fractionation of NIH3T3 cells in our laboratory revealed that endogenous NRX is predominantly cytosolic (39). NRX may shuttle between the cytosol and the nucleus, and regulatory mechanisms may control the subcellular localization of NRX [we further discuss this issue later (Subcellular localization of NRX)]. Kurooka *et al.* (88) showed that NRX possesses thiol-redox activity against insulin. We confirmed that the two conserved Cys residues are essential for oxidoreductase activity because the C205S/C208S mutant showed no oxidoreductase activity (39).

V. NRX IN VARIOUS SPECIES

A. Domain structure of vertebrate NRX

NRX proteins in *Homo sapiens* (human) and *Mus musculus* (house mouse) are composed of 435 amino acid residues and share 99% identity. NRX orthologues found in *Bos taurus* (cow), *Canis familiaris* (dog), *Rattus norvegicus* (rat), and *Macaca mulatta* (red monkey) are registered in databases. Further, the NRX orthologues found in many nonmammalian vertebrates, including *Xenopus laevis*, *Xenopus tropicalis* (clawed frog), *Gallus gallus* (chicken), and *Danio rerio* (zebrafish), also are registered.

A schematic of the NRX proteins of various species is shown in Fig. 5. All NRX proteins possess a WCPPC motif in the TRX-like domain. However, the amino acid sequence of the TRX-like domain does not share high homology with TRX (25% identity between human TRX1 and human NRX). In addition, no residue corresponds to Asp26 in human TRX1 that aids in the deprotonation of the catalytically active Cys residue (GRX and several other TRX family members also do not possess this acidic residue). The TRX-like domain of NRX is rather more similar to that of tryparedoxin (TryX, also known as TPX), an oxidoreductase first identified in the trypanosomatid *Crithidia fasciculate* [*Crithidia fasciculate* TryX shares 42% identity with human NRX1, but only marginal homology (~20%) with human TRX1 or GRX1] (128). Trypanosomatids are parasite protozoa that cause trypanosomiasis in various species. Human trypanosomiasis can be divided into two ma-

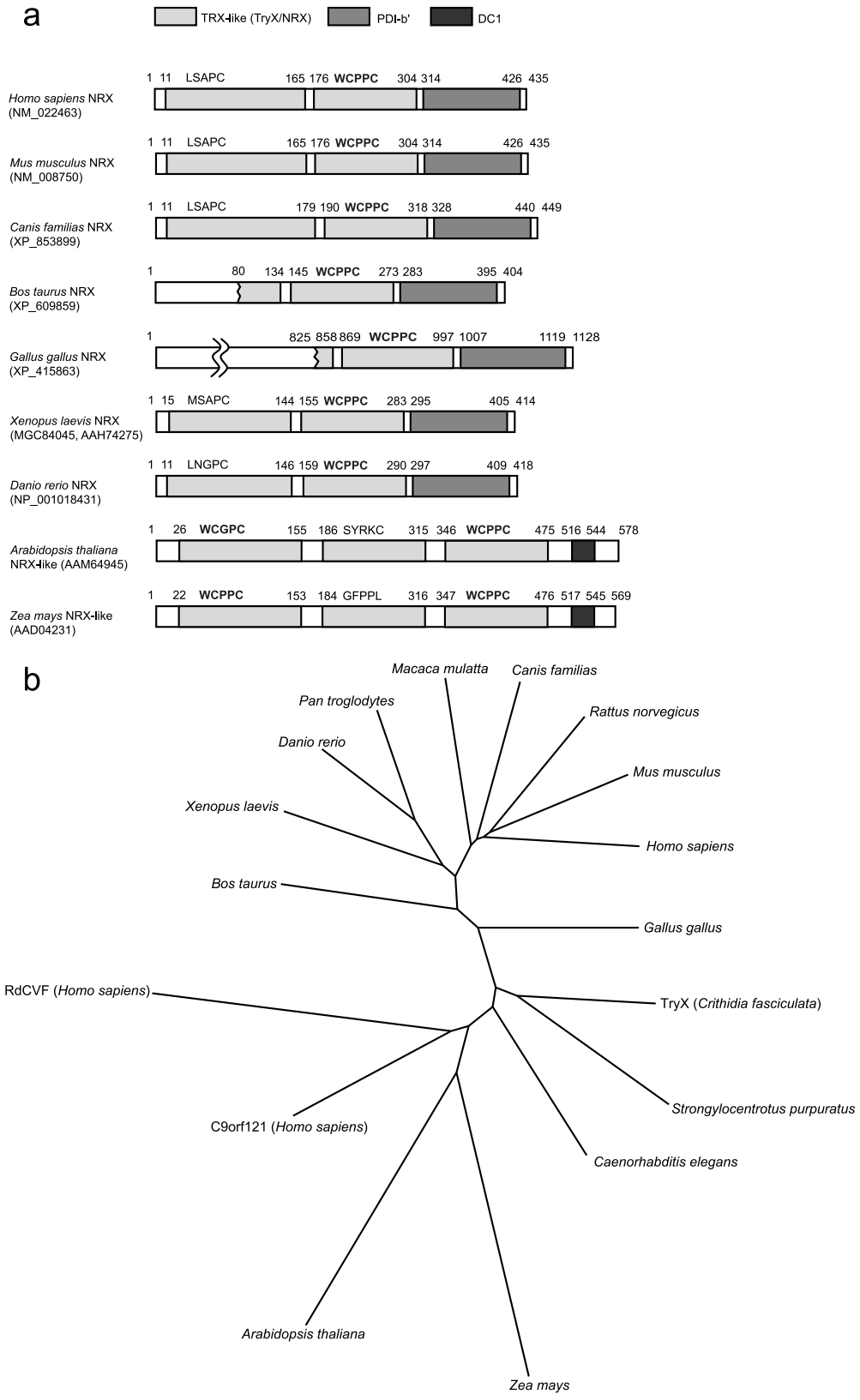

FIG. 5. NRX protein in various species. (**a**) Domain structures of NRX in various species. TRX-like (TryX/NRX), tryparedoxin (TryX)-homologous region; PDI-b′, region homologous to PDI-b′ domain; DC1, DC1 domain (similar to zinc-finger domain). Amino acids of the predicted catalytic motifs are denoted in bold, and putative inactive motifs are denoted in regular font. (**b**) Dendrogram of NRX in various species. The accession numbers of NRX, RdCVF, C9orf121, and TryX used to create this figure are as follows. *Homo sapiens* NRX, NM_022463; *Mus musculus* NRX, NM_008750; *Rattus norvegicus* NRX, XP_340858; *Canis familias* NRX, XP_853899; *Macaca mulatta* NRX, XP_001085223; *Pan troglodytes* NRX, XP_001152479; *Danio rerio* NRX, NP_001018431; *Xenopus laevis* NRX (MGC84045), AAH74275; *Bos taurus* NRX, XP_609859; *Strongylocentrotus purpuratus* NRX-like, XP_001181924; *Arabidopsis thaliana* NRX-like, AAM64945; *Zea mays* NRX-like, AAD04231 T20D4.7; *Caenorhabditis elegans* T20D4.7 protein (TryX-like domain-containing), NP_503954; *Crithidia fasciculate* TryX, AAC72299; *Homo sapiens* C9orf121, Q5VZ03; *Homo sapiens* RdCVF (TRX-like 6), AAH14127.

jor subtypes—sleeping sickness and Chagas disease—together, these kill thousands of people each year (6). TryX is an essential component of redox homeostasis in trypanosomatids (33, 86, 166). The redox-reaction cascade in trypanosomatids is composed of NADPH, trypanothione reductase (TR), trypanothione, TryX, and TryX peroxidase (see Fig. 1c). TR is moderately homologous to glutathione reductase and to TRX reductases (the *Crithidia fasciculate* TR shares 35% and 33% identity between with human glutathione reductase and human TrxR1, respectively). Trypanothione [N^1,N^8-bis(glutathionyl)-spermidine] is a metabolite first discovered in trypanosomatids (Fig. 6); it is a double-glutathione compound linked by a polyamine (spermidine) and can reduce TryX effectively. Trypanothione is a unique compound in trypanosomatids, and no reports show the existence of trypanothione in other organisms (86) [a report shows the existence of a very similar compound, glutathionylspermidine, a spermidine plus a single glutathione compound, in *Escherichia coli* (174)]. TryX peroxidase is homologous to mammalian Prx (59% identity between *Crithidia fasciculate* TryX peroxidase and human Prx1). Whether mammalian NRX also functions in a redox cascade similar to that in which TryX participates remains unknown. Even if such a redox cascade exists, the peptide corresponding to trypanothione remains to be characterized. No reports exist of mammalian trypanothione (therefore, the trypanothione cascade has been considered a good target for new drugs against trypanosomiasis); the most likely candidate is glutathione. This point should be a major focus of future research on mammalian NRX.

The N-terminal region of mammalian NRX possesses another TryX-homologous region with less homology to TryX, but considerably higher homology than that to TRX (29% identity to TryX, and almost no identity to TRX). In this N-terminal TryX-like region, the WCPPC motif is altered to LSAPC. As described earlier, the first Cys residue in this motif is thought to be important for TRX oxidoreductase activity. A deletion mutant of mouse NRX lacking the N-terminal TryX-like region possesses oxidoreductase activity against insulin (88) and binds to its binding partners [Dishevelled and $PP2A_C$, at least *in vitro* (39, 93)]. Therefore, it may be critical for a physiologic function of NRX, just not for the potential functions studied to date. Another conserved Cys residue exists a few amino acids before LSAPC to give a CXXXXXC motif, and other proteins involved in thiol-disulfide exchange reactions do not just have CXXC motif [*e.g.*, Ero1 has both conserved CXXC and CXXXXC motifs (36)]. Further, NRX N-terminal domain is well conserved among species and is found in most higher species (human, monkey, dog, mouse, rat, *Xenopus*, zebrafish, and corn). It should be noted that NRX orthologues in chicken and cow possess only the latter portion of the N-terminal TryX-like region (they both possess a complete central TryX-like domain). These sequences are registered in databases, but a detailed characterization has yet to be performed. In addition, chick NRX has a long N-terminal sequence before its truncated N-terminal TryX-like domain. This long N-terminal region of chick NRX is not found in other NRX orthologues, does not possess any known domains, and its function is unknown. Whether chick and cow NRXs possess a partially truncated TryX-like domain or these sequences only reflect sequencing errors remains to be determined. Nevertheless, further research on this mysterious N-terminal TryX-like domain in NRX may shed light on the importance of this region.

There are no reports regarding the 3D structure of NRX. However, several reports regard the 3D structure of TryX (1–3, 55, 87, 116). Although the amino acid sequences of TryX and

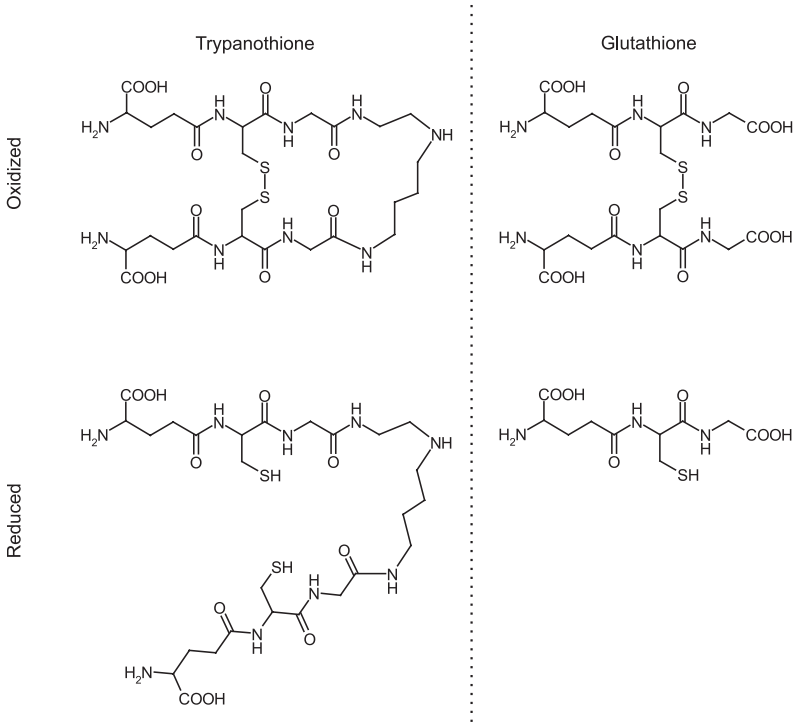

FIG. 6. Structures of glutathione and trypanothione. *Left*, trypanothione. *Right*, glutathione. *Upper row*, oxidized form. *Lower row*, reduced form. Trypanothione is a formed from two glutathiones linked with spermidine.

TRX do not share high sequence homology, these proteins have similar structures. TRX has a characteristic α/β fold composed of five twisted β-strands and four surrounding α-helices (63). TryX has seven β-strands and four α-helices, and among these secondary structures, five β-strands and four α-helices show a 3D alignment structurally similar to that of TRX (2). The remaining two β-strands exist outside the TRX-like fold. The function of these two β-strands, which do not exist in TRX, in the TryX oxidoreductase reaction has yet to be determined.

The C-terminal region of mammalian NRX contains an acidic region that resembles the b′ domain of PDI proteins. The PDI-b′ domain does not possess catalytic activity, but it is required for substrate recognition (81). The role of the PDI-b′–like domain of NRX is unknown, but this domain may participate in the substrate specificity of NRX oxidoreductase activity.

B. Other TryX-like domain-containing proteins

In addition to NRX, several mammalian proteins contain TryX-like sequences (Fig. 7). Léveillard *et al.* (98) identified the rod-derived cone viability factor (RdCVF), expressed by photoreceptors, which enhances the viability of chicken rod cone cells (98). They showed that recombinant RdCVF can rescue *rd1* knockout mouse–derived rod cone cells from degeneration. The *rd1* gene is involved in retinitis pigmentosa, a retinal disease that causes rod cone degeneration and leads to blindness (11). This gene encodes a cGMP-specific phosphodiesterase. For details and other causative genes for retinal diseases, please visit http://www.sph.uth.tmc.edu/RetNet/sum-dis.htm). RdCVF possesses a single TryX-like domain (32% identity to the central TryX-like domain in NRX) and is also called TRX-like 6 (Txnl6). RdCVF is expressed exclusively in the retina but is expressed to a lesser extent in *rd1*-knockout mice. The mechanism whereby RdCVF enhances rod cone cell viability remains unknown. RdCVF possesses a putative catalytic motif (ACPQC). However, no oxidoreductase activity has been reported. Only the short form of RdCVF (109 amino acids) was assessed for catalytic activity; the longer variant (212 amino acids) may possess activity. Another possibility is that RdCVF requires cofactors for oxidoreductase activity. A report suggests that antioxidants decrease the degeneration of rod cone cells in *rd1*-deficient mice (85). Future studies will reveal the detailed mechanism of RdCVF function as a rod cone survival factor and the relation with its potential oxidoreductase activity.

Chromosome 9 open reading frame 121 (C9orf121) protein is another protein containing TryX-like domain. The function of C9orf121 has not been reported. It is homologous to the central TryX-like domain of NRX (34% identity), and the corresponding amino acid sequence for the oxidoreductase catalytic motif is RCAPS. Whether C9orf121 possesses another Cys residue that can fulfill the role played by the second Cys residue of the TRX catalytic motif {as in the case of another second Cys mutated TRX family protein GRX5 [its catalytic motif is QCGFS (176)]} remains to be determined. As written in the previous section, GRX5 is important for the generation of Fe-S clusters (150, 193). It may be interesting to examine whether C9orf121 has a related function.

NRX, RdCVF, and C9orf121 are conserved among various species; the sequences of the orthologs of RdCVF and C9orf121 in mouse, rat, dog, cow, monkey, *Xenopus*, chicken, and zebrafish are registered. It should be stressed that these three TryX-like domain–containing proteins do not share their active motifs; the motifs of NRX, RdCVF, and C9orf121 are WCPPC, ACPQC, and RCAPS, respectively. All these motifs also differ from WCGPC, the active motif of TRX. However, when we grouped TRX family members by sequence homology, we found that these proteins are clustered in the same group (see Fig. 3). Therefore, some unknown common role of these proteins may exist, and they can be considered to form an "NRX (or TryX-like) subfamily," even if their active motifs are different. Nevertheless, further research is required.

C. NRX-like proteins in plants

Laughner *et al.* (91) reported maize (*Zea mays* L) NRX. Maize NRX (zNRX hereafter) possesses three TryX-like domains, and each is similar to the two TryX-like domains of mammalian NRX (see Fig. 5); the most homologous TryX-like domain in zNRX shares 49% identity with the central TryX-like domain of human NRX. The N-terminal and C-terminal TryX-like domains of zNRX have WCPPC motifs, and zNRX possesses significant oxidoreductase activity against insulin, although it is less than that of *Escherichia coli* TRX. Whether both (or either) of the putative TryX-like domains in the zNRX protein exerts an oxidoreductase activity is not clarified. zNRX and mammalian NRXs have different domain structures in their C-terminal regions. Mammalian NRX has a domain that resembles the PDI-b′ domain. However, zNRX has no such domain and instead has a divergent C1 (DC1) domain (see Fig.

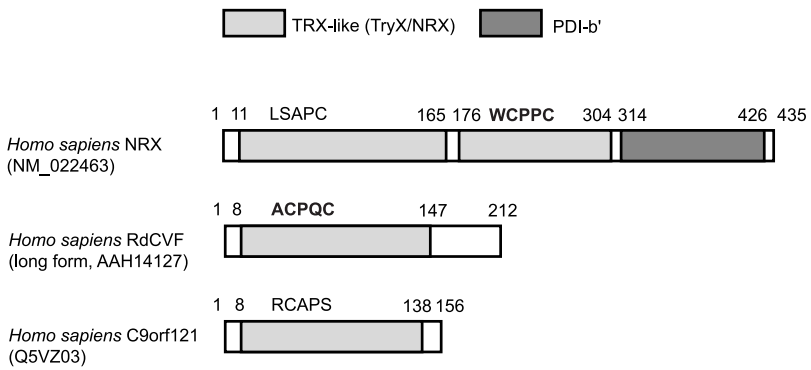

FIG. 7. Tryparedoxin (TryX)-like proteins in mammals. TRX-like (TryX/NRX), tryparedoxin (TryX) homologous region; PDI-b′, region homologous to the b′ domain of PDI; DC1, DC1 domain (similar to the zinc-finger domain). The amino acids of the predicted catalytic motif are denoted in bold, and putative inactive motifs are denoted in regular font. For RdCVF, we show the longest form available in the database.

5). The DC1 domain binds zinc, and the sequence is similar to that of zinc-finger domains. Immunostaining of maize kernel sections with anti-zNRX antibody showed that zNRX is localized to the nucleus on the kernels on day 13 after pollination. Whether the C-terminal zinc finger–like DC1 domain of zNRX affects its subcellular localization has yet to be determined. Western and Northern blot analyses revealed that zNRX is abundant in kernels but is expressed in lower levels in leaves, epicotyls, stems, and roots. In addition to zNRX, NRX-like proteins are found in *Quercus suber* (cork oak), *Cucurmis melo* (muskmelon), *Oryza sativa* (rice), and *Arabidopsis thaliana* (arabidopsis, thale cress). The function of these plant NRX-related proteins is still unknown, and because they have different domain structures from mammalian NRX, it may be better to recognize them as different proteins of the same family. Because zNRX is reported to localize in the nucleus, plant NRX-related proteins may regulate transcriptional activity as is reported for mammalian NRX [discussed in the later sections (53)]. Another possibility is that as NRX is a TryX-homologous protein and that plant NRX-related proteins may be involved in metabolite detoxification by using glutathione as a hydrogen donor. These speculations should be clarified by future studies.

D. TryX-like domain-containing proteins in nematodes

Several NRX (TryX)-like proteins in *Caenorhabditis elegans* are registered in a database (WormBase: www.wormbase.org). The protein most similar to human NRX is C32D5.8, with 42% identity. The T20D4.7, K02H11.6, Y52E8A.3, R05H5.3, T28A11.13, and F29B9.5 proteins also show similarity to human NRX1. Among these NRX-related proteins, C32D5.8, T20D4.7, R05H5.3, T28A11.13, and F29B9.5 possess the WCPPC motif (the other two possess WCGPC). All of these proteins bear a single TryX-like domain (which is quite different from the mammalian NRX domain structure), and the existence and function of oxidoreductase activity are unknown. Interestingly, the RNAi of C32D5.8, R05H5.3, and F29B9.5 showed defects (embryonic lethality, larval arrest, and sickness), suggesting that these proteins containing TryX-like domains may play essential roles.

Redox homeostasis is known to control aging in various species. TRX family proteins are implicated in the aging process (73, 117, 119, 203, 204). Essers *et al.* (31) reported that BAR-1 (*Caenorhabditis elegans* β-catenin) interacts with FOXO (DAF-16) and regulates longevity (31). As is discussed in more detail later, NRX regulates the Wnt/β-catenin pathway in mammalian cells (39). Proteins containing TryX-like domains in *Caenorhabditis elegans* may regulate lifespan by controlling FOXO *via* β-catenin in a redox-dependent manner.

VI. EXPRESSION PATTERNS OF NRX IN MAMMALS

The tissue distribution of NRX mRNA in adult mice was reported by Kurooka *et al.* (88). Northern blotting revealed positive signals for NRX mRNA in all tissues analyzed, including the skin, brain, heart, liver, kidney, lung, testis, skeletal muscle, thymus, and spleen, with predominant expression in the skin and testis. To examine the expression during development, they also performed whole-mount *in situ* hybridization. In E9.5 and E10.5 embryos, NRX mRNA was found in various sites including somites, dorsal root ganglia, branchial bars, and some parts of the brain. Strong signals were also observed in limb buds. In E11.5 embryos, the expression became restricted to the distal ends of the limb buds and to the anterior region of the tail. The function of NRX in these organs has not been characterized. However, the embryonic expression pattern strongly resembles that of the segment polarity gene *dishevelled 3* (183). We discuss the relation between Dishevelled and NRX in detail in later sections.

VII. REGULATION OF NF-κB, AP-1, AND CREB REPORTER ACTIVITIES BY NRX EXPRESSION

To determine the differences between NRX and other TRX family proteins, Hirota *et al.* (53) compared NRX, TRX, and GRX by ectopically expressing these proteins in HEK293 cells and monitoring the reporter activities of several transcription factors, including NF-κB, activation protein-1 (AP-1), and cyclic-AMP response element–binding (CREB) protein, with and without treatments with various compounds [TNF-α, phorbol-12 myristate 13-acetate (PMA), and forskolin (Table 2) (53)].

NRX overexpression enhanced forskolin-stimulated CREB reporter activity more than the activities of the other two compounds. The ectopic expression of TRX or GRX also upregulated CREB reporter activity in forskolin-treated cells, but NRX expression increased the activity by more than twofold when compared with TRX or GRX expression. This upregulation of

Table 2. Effect on NF-κB, AP-1, and CREB by NRX Expression

	CREB	*AP-1*	*NF-κB (conventional reporter assay)*	*NF-κB (transactivation assay & IκB phosphorylation)*
TRX	↑	↑	↓ (TNF-α activation), ↑ (NIK activation)	↓
GRX	↑	↑	↑	↓
NRX	↑ ↑	↑	↑	—

CREB reporter activity by NRX expression was confirmed in cells cotransfected with protein kinase A (PKA; it activates CREB by direct phosphorylation) and in reporter assays with GAL4-fused CREB proteins. How NRX activates CREB reporter activity is unknown. The interaction between CREB and the CRE DNA fragment has been reported to increase in response to reduction (41). The simplest scenario is that NRX reduces CREB *via* its oxidoreductase activity, thus enhancing its affinity for CRE.

In AP-1 reporter assays, NRX-expressing cells showed moderate enhancement of AP-1 reporter activity when they were treated with PMA or cotransfected with MEKK (MAPKKK); this enhancement was stronger than that observed in TRX- or GRX-expressing cells. For the analysis of the NF-κB pathway, luciferase reporter assays with vectors expressing GAL4-fused p65/RelA (a transcription factor in the NF-κB pathway) were used to assess the direct activation of p65/RelA. This method has an advantage in that it can monitor only the activation of the transcription factor of interest, but not that of other factors or the DNA-binding affinity of the transcription factor being studied. TRX- or GRX-expressing cells showed significant suppression of TNF-α–induced activation of reporter activity, whereas NRX expression showed no significant effect. An immunoblot analysis with anti-phospho-IκB antibody revealed that the TNF-α–induced phosphorylation of IκB was not significantly blocked by NRX expression, but it was clearly reduced by TRX or GRX expression. Therefore, it appears that NRX does not affect p65/RelA activation induced by TNF-α stimulation. However, luciferase assays with the conventional construct used for examining the NF-κB pathway (pNF-κB-Luc) showed that NRX overexpression enhances the reporter activity of cells stimulated by TNF-α treatment or by NF-κB–inducing kinase (NIK) overexpression. Thus, NRX appears to stimulate the transcriptional activity of NF-κB not through p65/RelA but through other unidentified components.

VIII. NRX SUPPRESSES WNT/β-CATENIN SIGNALING IN A REDOX-DEPENDENT MANNER THROUGH DISHEVELLED (DVL)

A. Summary of Wnt signaling pathway

In 1973, Sharma (159, 160) identified a mutant of *Drosophila melanogaster* and named it Wingless, based on its lack of wings. The *Wingless* gene encodes a secreted protein that stimulates a signaling pathway that is evolutionarily conserved from nematodes to mammals (82). The orthologous mammalian gene was first identified as *Int-1*, which was activated and induced the formation of mammary gland tumors in mice on infection with mouse mammary tumor virus (131). When it became clear that the *Int-1* gene product was the mammalian homologue of Wingless (147), the name "Wnt" was coined as a combination of Wingless and Int. The mammalian Wnt signaling pathway, which is produced by various Wnt ligands (19 human Wnt genes are known) and other extracellular and intracellular molecules, plays essential roles in early morphogenesis and stem cell maintenance (20, 120, 125, 143). Genetic and biochemical studies have revealed the basic Wnt signaling pathway in mammalian cells (Fig. 8; for further details please see "The Wnt homepage" http://www.stanford.edu/~rnusse/wntwindow.html). In brief, the Wnt ligand binds to the Frizzled (Fzd) receptor, and through

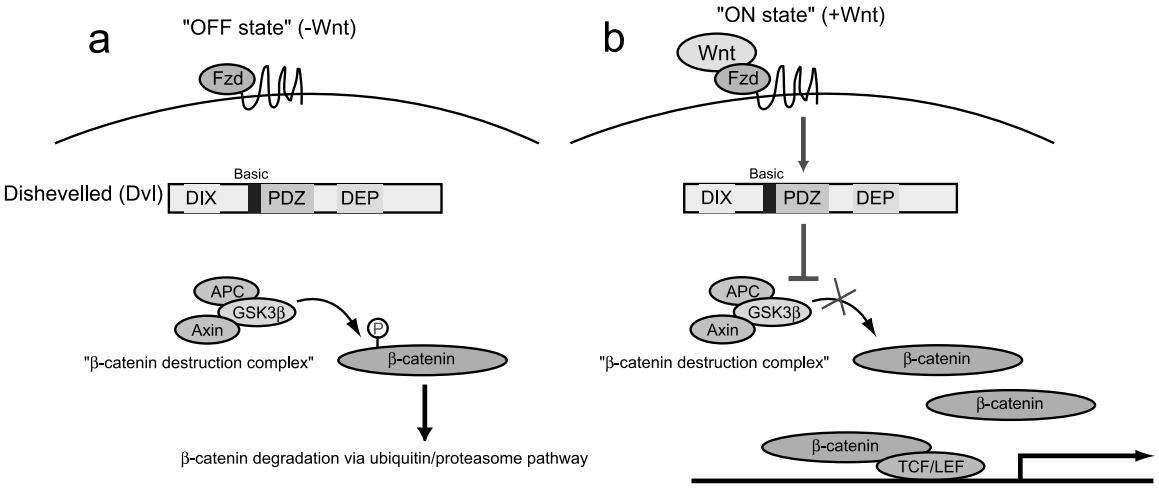

FIG. 8. Model of the mammalian Wnt/β-catenin pathway. (**a**) OFF state (without Wnt ligand). Without the Wnt ligand, the "β-catenin destruction complex," composed of Axin, APC, and GSK3β is active, and GSK3β can efficiently phosphorylate cytosolic β-catenin. Phosphorylated β-catenin is readily degraded *via* the ubiquitin/proteasome pathway, and thus the cytosolic level of β-catenin is kept very low. (**b**) "ON state" [Wnt ligand bound to its receptor, Frizzled (Fzd)]. When Wnt binds to its receptor Fzd, the signal is transmitted through the adaptor protein Dishevelled (Dvl). Dvl inhibits the β-catenin destruction complex, and the phosphorylation of β-catenin is suppressed. The nonphosphorylated form of β-catenin is no longer a target of the ubiquitin/proteasome pathway, and it accumulates. Some of the accumulated β-catenin moves into the nucleus, binds to the transcription factor TCF/LEF, and activates the transcription of various target genes.

the adaptor protein Dishevelled (Dvl), induces the cytosolic accumulation of β-catenin by inhibiting the β-catenin destruction complex composed of Axin, adenomatous polyposis coli (APC), and a serine/threonine kinase, glycogen synthase kinase 3 beta (GSK3β. The accumulated β-catenin then migrates into the nucleus and activates the transcription factor T-cell factor/lymphoid enhancer factor (TCF/LEF).

The Wnt/β-catenin pathway is crucial not only for early morphogenesis but also for stem cell maintenance, and it is widely accepted that the misregulation of Wnt/β-catenin signaling leads to tumorigenesis (20, 120, 143). In addition to *Int-1*, *APC* is known to be deeply involved in tumorigenesis. *APC* is causative in familial adenomatous polyposis (FAP), an autosomal disorder that induces the formation of multiple colorectal polyps and frequently leads to colorectal tumors (80). Studies have revealed that Wnt/β-catenin signaling is hyperactivated in many tumors, as determined by the accumulation of β-catenin in the cytosol or in the nuclei of tumor cells. Alterations in the genes encoding APC, β-catenin, and Axin have been reported. Recent reports indicate that the expression of secreted Fzd-related protein (sFRP), a secreted antagonist of the Wnt ligand, is epigenetically silenced in tumor cells (113, 151, 173, 201). Surprisingly, restoration of the expression of sFRP suppresses Wnt/β-catenin signaling and induces apoptosis, even in cells containing mutations of downstream *APC* or *β-catenin* genes. These reports suggest that aberrant activation of Wnt/β-catenin signaling cannot be explained simply by genetic alterations and that this pathway is hyperactivated by multiple complex mechanisms.

B. Known function of Dishevelled (Dvl)

A novel mutant called *dishevelled* (*dsh*), which causes a segment polarity phenotype, was identified in *D. melanogaster* by Perrimon *et al.* (137) in 1987. Subsequently, Dsh was shown to be an important component of the canonic Wnt signaling pathway in *Drosophila* (83, 130, 162).

The mammalian orthologue of Dsh, Dishevelled (Dvl), was identified in 1994 (172), and three mammalian Dvl genes are known, *Dvl1-3* (139, 172, 183). Dvl is crucial for transduction of the Wnt/β-catenin signal from the Wnt receptor Fzd to downstream components. Genetic studies in *Drosophila* have indicated that *Dsh* is essential not only in the so-called canonic Wnt/β-catenin pathway but also in the planar cell polarity (PCP) pathway (10). The PCP pathway regulates cell orientation *via* a serine/threonine kinase JNK in a manner independent of β-catenin accumulation. In mammalian cells, it has been shown that the ectopic expression of Dvl activates JNK (100, 121). Thus, Dvl is essential for both Wnt/β-catenin signaling and JNK activation, and it plays a pivotal role in delivering the signal from various Wnt ligands to these pathways. However, the precise molecular mechanism by which Dvl delivers these signals to downstream components remains to be determined.

Dvl possesses three conserved domains: the N-terminal Dvl, Axin (DIX) domain (12), the central postsynaptic density-95 (PSD-95), Discs large (Dlg), zona occludens (ZO1) (PDZ) domain (in the case of Dvl, an adjacent basic region is in the N-terminal side of the PDZ domain, and it is thus frequently called the basic-PDZ domain) (196), and the C-terminal Dvl, EGL-10, pleckstrin (DEP) domain (140). The DIX and PDZ domains are known to be involved in the Wnt/β-catenin pathway, and the PDZ and DEP domains appear to be involved in the Wnt/JNK pathway.

Interestingly, *Dvl1*-knockout mice show abnormalities in social behavior (102). How *Dvl1* deletion leads to such defects remains largely unknown, but it is reported that Dvl1 regulates neuronal development. Indeed, hippocampal neurons obtained from *Dvl1*-knockout mice show defective dendrite development (153). It appears that such defects are not directly reflected at the gross anatomic level, but may underlie abnormalities in higher cognitive functions, such as social behavior. Mice lacking both *Dvl1* and *Dvl2* show neural tube–closure defects (43).

C. Identification of NRX as a novel Dvl-binding protein

Despite extensive research on the Wnt signaling pathway, the molecular mechanism of its signal transduction is far from understood. One major question is how the adaptor protein Dvl transmits the signal from the Wnt receptor Fzd to downstream effectors ("β-catenin destruction complex" composed of Axin, GSK3β, and APC). Therefore, we searched for novel Dvl-interacting proteins to clarify the molecular mechanism of the Wnt/β-catenin pathway and identified NRX as the most abundant protein to coprecipitate with Dvl (39). The *in vivo* interaction between Dvl and NRX was confirmed by immunoprecipitation experiments on endogenous proteins (39). NRX binds directly to the central basic-PDZ domain, but it does not bind to the basic region or to the PDZ domain alone.

D. NRX is a selective inhibitor of Wnt/β-catenin pathway

The overexpression of NRX induces a significant band shift of Dvl, reflecting a change in the phosphorylation status. Numerous reports have shown that the phosphorylation status of Dvl correlates with the activation of the Wnt/β-catenin pathway (198), suggesting that NRX regulates the signal-transducing ability of Dvl. Indeed, NRX expression suppresses Wnt/β-catenin signaling stimulated by Wnt ligands or by Dvl overexpression, and the RNAi of endogenous NRX induces upregulation of the Wnt/β-catenin pathway (39). This inhibitory effect of NRX overexpression is not observed in transfectants of the constitutively active form of β-catenin, and NRX-RNAi–induced activation of the Wnt/β-catenin pathway is clearly cancelled by the expression of the dominant-negative form of the Wnt/β-catenin signal transcription factor TCF/LEF. Therefore, we concluded that NRX negatively regulates the Wnt/β-catenin pathway at the level of Dvl. How NRX alters the phosphorylation status of Dvl is not clear. Numerous kinases, including casein kinase (CK) Iε(138), CK-II (192), and Par IB (170), have been reported to modulate Dvl phosphorylation directly. In addition, several phosphatases, including protein phosphatase (PP) 2A (142, 157) and PP2Cα (169), have been shown to regulate the activity of the Wnt/β-catenin pathway. Interestingly, it was recently reported that PP2A interacts directly with NRX (93), which is discussed later. It is possible that NRX alters the phosphorylation status of Dvl by modulating the function of these kinases or phosphatases or both.

We showed that NRX does not inhibit JNK activation by Dvl overexpression in kinase assays (39). Therefore, NRX may be a selective inhibitor of the Wnt/β-catenin pathway. We found that NRX competes with frequently rearranged in advanced T-cell lymphomas (Frat) for Dvl binding *via* the blockade of the basic-PDZ domain of Dvl. It is known that Frat [also known as GSK3β binding protein (GBP) in *Xenopus laevis*] also interacts directly with the basic-PDZ domain of Dvl to specifically activate the Wnt/β-catenin pathway (99, 205). In addition to Frat/GBP, numerous Dvl-interacting proteins are found [reviewed in (190)]. It is unclear which of these competes with NRX. In this regard, casein kinases (CKs) are interesting candidates. CKIε is reported to phosphorylate Dvl and to activate the Wnt/β-catenin signaling (138). In addition, CKIε enhances the interaction between Dvl and Frat/GBP (50). CKII is also known as Dvl-phosphorylating kinase (192). The inhibition of interaction with these kinases by the presence of NRX would result in the downregulation of Dvl phosphorylation. Future research will shed light on the details of the molecular mechanism underlying the role of NRX as a negative regulator of Wnt/β-catenin signaling.

E. NRX serves as a negative regulator of Wnt/β-catenin pathway in a redox-dependent manner

The interaction between purified recombinant Dvl and NRX proteins is strengthened by treatment with dithiothreitol (DTT) and is weakened by treatment with H_2O_2. Thus, the Dvl/NRX association is regulated in a redox-dependent manner. This is supported be the fact that the intracellular colocalization of Dvl and NRX is suppressed by H_2O_2, and the Wnt/β-catenin pathway is upregulated by H_2O_2. Dvl localization plays an important role in Wnt/β-catenin signal transduction (32). This redox-dependent association/dissociation and activation of signaling is similar to that of the TRX/ASK1 system [(155) and Fig. 9]. When cells are challenged with H_2O_2, TRX forms an intramolecular disulfide bond, and the resulting conformational change allows the dissociation of ASK1 from TRX. ASK1 then self-activates and transduces the signal to downstream effector molecules.

We have shown that NRX functions as a redox-sensor to regulate Wnt/β-catenin signaling. The Wnt/β-catenin pathway is activated in an ROS-dependent manner, possibly because cells may use this pathway to promote proliferation. The Wnt/β-catenin pathway stimulates cell proliferation *via* the transcriptional activation of genes such as *c-Myc* and *Cyclin D1* (48, 179). Growth factors are known to stimulate the intracellular generation of ROS (171), which is essential for the proliferative response, through various signaling intermediates (34, 145). Many reports show high levels of ROS in tumor cells (182). Therefore, the ROS-induced activation of Wnt/β-catenin signaling may contribute to cell proliferation. NRX-RNAi cells show a higher proliferation rate. By the transfection of the constitutively active form of MEK or Ras, NRX-RNAi cells form more foci compared with control RNAi cells (39).

Activation of the Wnt/β-catenin pathway also protects cells from apoptosis (16, 47, 107). In addition, reports suggest that ROS exert an antiapoptotic effect on cells (67, 194). Wong and Goeddel (194) showed that TNF-α stimulation, which causes a massive generation of ROS, induces the expression of manganese superoxide dismutase (MnSOD), an enzyme that scavenges $O_2^{\cdot -}$ and helps prevent cell death by apoptosis. Therefore, it is possible that the ROS-dependent activation of the Wnt/β-catenin pathway helps prevent cells challenged by a moderate level of ROS from undergoing unnecessary apoptosis. Huang *et al.* (66) performed a microarray analysis of HeLa cells treated with RNAi against β-catenin (thus, Wnt/β-catenin signaling is downregulated, and cells are prone to apoptosis) and identified several upregulated genes, including apoptosis-antagonizing transcription factor (AATF) and Fas-associated

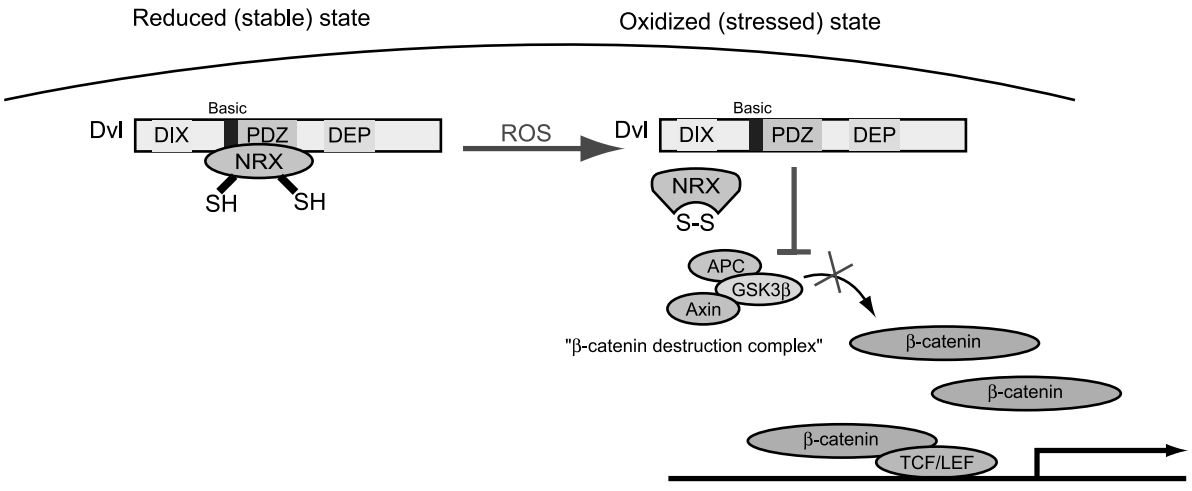

FIG. 9. Model of redox regulation of the Wnt/β-catenin pathway by the Dvl/NRX complex. In the stable state, NRX is reduced. Reduced NRX binds strongly to Dvl and blocks downstream Wnt/β-catenin signaling. When ROS oxidize NRX, NRX dissociates from Dvl, and free Dvl can now transduce the signal to downstream effectors, resulting in the activation of Wnt/β-catenin signaling.

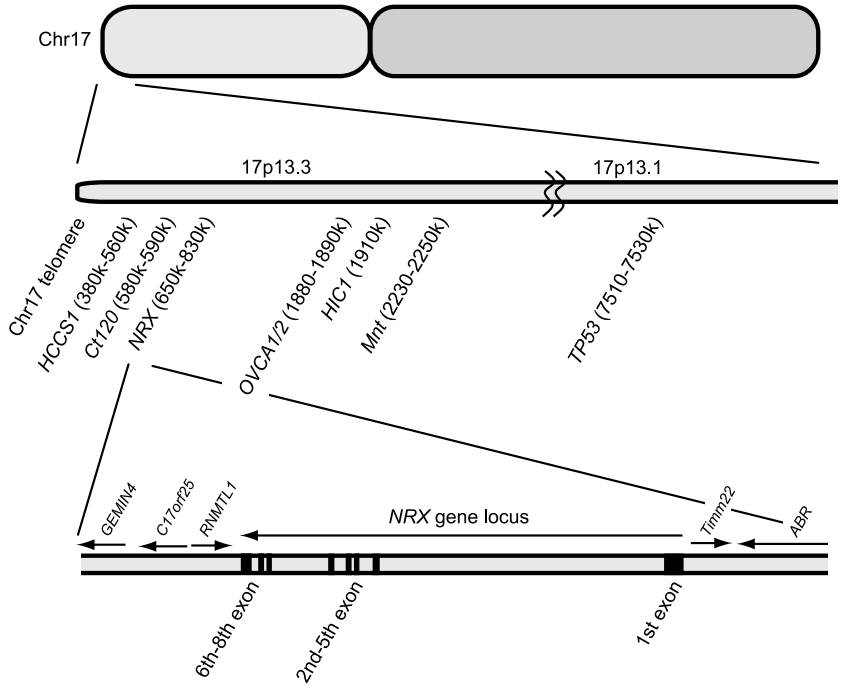

FIG. 10. Schematic of the distal end of human chromosome 17. The enlarged view shows the genomic region of the distal end of the short arm of human chromosome 17 to the *TP53* (gene encoding p53) locus. The positions of tumor-related gene candidates are also shown.

protein with death domain (FADD), that accelerate apoptosis (66). It would be interesting to determine whether the expression of these genes is downregulated in response to moderate levels of ROS.

IX. THE *NRX* GENE

The structure of the human *NRX* gene is shown in Fig. 10 (lower panel). The human (and mouse) *NRX* gene contains eight exons. Between the first and second exons, a large gap of ~150 kb is seen. The other seven exons are located closer together. No reports exist of splice variants of NRX. Kurooka *et al.* (88) reported two NRX transcripts (a major transcript of ~2.2 kb, and a minor transcript of ~2.4 kb) by Northern blot (88). The promoter and enhancer regions of the *NRX* gene have not been identified.

The genes surrounding the *NRX* gene are well conserved between mouse and human. The *translocase of inner mitochondrial membrane 22 homolog (Timm22)* and *active breakpoint cluster region (BCR)-related (Abr)* genes are located next to the human *NRX* gene at the proximal side. *RNA methyltransferase like 1 (Rnmtl1)*, *C17orf25*, and *gem (nuclear organelle)-associated protein 4 (GEMIN4)* genes are located at the distal side. This gene alignment is clearly the same as that in the murine *NRX* gene. In addition, the *Tp53* (*Trp53* in mouse), *ovca1*, and *Hic1* genes are located close to the *NRX* gene in mouse and human. At present, no direct evidence shows a relation between these genes.

The human *NRX* gene is located at 17p13.3, the distal end of chromosome 17 (see Fig. 10). Numerous reports show that the 17p13.3 region is frequently lost in human tumors, including breast tumors, hepatocellular carcinomas, cervical carcinomas, and medulloblastomas (21, 24, 27, 126). One candidate tumor-suppressor gene in the 17p13.3 region is *OVCA1* (15). *OVCA1* heterozygote mice spontaneously form tumors (14), suggesting that the *OVCA1* gene is critically involved in tumor formation. However, no ovarian epithelial tumors or breast tumors occur in these mice. Thus, other tumor-suppressor genes may be present in the 17p13.3 region. Zhao *et al.* (207) reported a human case of allelic loss of the distal region of the 17p13.3 region, which does not include the *OVCA1* locus, in hepatocellular carcinoma. It has also been reported that many sporadic breast tumors possess not only deleted regions around the *OVCA1* locus but also discontinuous deletions of the telomeric region of 17p13.3 (54).

In addition to *OVCA1*, several candidate tumor-related genes, including *hypermethylated in cancer 1 (HIC1)* (189), *hepatocellular carcinoma suppressor 1 (HCCS1)* (208), *Mnt* (23), *Ct120* (49), and *GEMIN4* [coding hepatocellular carcinoma–associated protein 1 (HCAP1)] (25), are localized to the 17p13.3 region. Among these, the most probable tumor-suppressor gene is *HIC1* (189). The *HIC1* gene is, as its name suggests, frequently hypermethylated in tumor cells. It was recently shown by Valenta and colleagues (186) that the gene product HIC1 attenuates Wnt/β-catenin signaling, similar to NRX. HIC1 binds directly to TCF4, a transcription factor of the Wnt/β-catenin pathway, and inhibits the β-catenin/TCF-driven transcription. Wnt/β-catenin signaling may not be fully activated by the mutation of a single component (173). An attractive hypothesis involves the inactivation of both the *HIC1* and *NRX* genes by chromosomal deletion, synergistically hyperactivating Wnt/β-catenin signaling and resulting in tumorigenesis.

Wnt/β-catenin signaling is frequently upregulated in many types of human tumors (120). For example, up to 60% of surgical specimens from breast tumors show positive β-catenin signals in the cytosol or nucleus or both (106). However, ge-

netic mutations in the components of the Wnt/β-catenin signaling pathway in these tumors are relatively rare (5, 133, 185). It is possible that ablation of the *NRX* gene hyperactivates Wnt/β-catenin signaling, thereby contributing to tumor formation. NRX-RNAi cells show an increased proliferation rate compared with control cells. It is reported that coactivation of the Wnt/β-catenin pathway and MAP kinase pathway can lead to cellular transformation by both increasing the amount of Cyclin D1 (148). Transfection of the constitutively activated mutant of Ras or MEK induces higher numbers of transformed foci in NRX-RNAi cells than in control cells (39). Thus, NRX appears to play an important role in preventing tumor formation. A detailed analysis of the status of the *NRX* gene in tumor samples will clarify this issue.

The promoter regions for the genes of many components of the Wnt/β-catenin pathway are hypermethylated in tumors (113, 151, 173, 201). This also may be the case for the *NRX* gene. Koinuma *et al.* (84) searched for hypermethylated regions in the genome of colorectal carcinoma cells, and a region of the *NRX* gene was identified. Further details of *NRX* gene hypermethylation are not described in this report, but it will be interesting to analyze the methylation status of the *NRX* gene in various tumors.

A genomic region around the mouse *NRX* gene is involved in type 1 and type 2 diabetes. Recently, Babaya *et al.* (4) examined the sequence of the *NRX* gene in several mouse strains (diabetes-resistant and diabetes-susceptible mice) but found no correlation between strain and sequence variability. Therefore, they concluded that *NRX* does not appear to be involved in diabetes. However, diabetes is not caused by a single gene mutation but rather by various acquired changes. Many reports indicate that ROS are involved in diabetes. Houstis *et al.* (65) reported that ROS-scavenging reagents and enzymes suppress insulin resistance induced by treatment with TNF-α or dexamethasone (65). The overexpression of TRX in pancreatic β-cells is reported to be protective against type 1 diabetes (64). Therefore, it may not be surprising if NRX, which belongs to the TRX family and possesses oxidoreductase activity similar to TRX, is involved in diabetes. In addition, many reports suggest the relation between the Wnt signaling pathway and diabetes or underlying adipogenesis (38, 42, 152). Taken together, we think it is still possible that NRX is linked to diabetes. Even if no genetic alteration in the *NRX* gene occurs, epigenetic and posttranscriptional regulations may contribute to the association with diabetes.

X. POSSIBLE ROLE OF NRX IN EARLY DEVELOPMENT

In *Xenopus laevis*, the NRX orthologue MGC84045 encodes a protein with 77% identity to the human NRX1 protein. An injection of antisense morpholino oligonucleotide (MO) against the MGC84045 mRNA into the animal pole region of fertilized *X. laevis* eggs results in significant defects in head morphology (39). Wnt/β-catenin signaling regulates anterior–posterior axis formation, and the perturbation of Wnt/β-catenin signaling in the animal pole region of *X. laevis* eggs destroys the anterior–posterior axis [Fig. 11 and (79, 115)]. Wnt/β-catenin signaling is aberrantly upregulated in NRX-MO–injected eggs, and the resulting head defect is rescued by coinjection of the mRNA for negative regulators of Wnt/β-catenin signaling such as GSK3β. These results clearly show that endogenous NRX regulates Wnt/β-catenin signaling and determines the anterior–posterior axis formation in *X. laevis*.

To date, no other reports exist on the involvement of NRX in early development. It is reported that in mice, NRX is expressed in somites, dorsal root ganglia, branchial bars, some regions of the brain, and limb buds (88). Interestingly, the expression pattern of the *Dvl3* mRNA in mouse embryos shows strong similarities to that of the *NRX* mRNA (183). In E10.5 embryos, both *Dvl3* and *NRX* mRNAs are expressed strongly in the aforementioned areas (somites, dorsal root ganglia, branchial bars, brain, and limb buds). Therefore, it is likely that NRX plays some role in these regions through the regulation of Wnt/β-catenin signaling. We have confirmed that similar to Dvl1, Dvl2, and Dvl3 can also bind efficiently to NRX (Funato *et al.,* unpublished observations).

Limb bud formation is regulated by several signaling pathways, including those involving fibroblast growth factor (Fgf), Hedgehog (Hh), and Wnt/β-catenin (13, 127, 200). Multiple Wnt ligands, secreted inhibitors of Wnt proteins, and Fzd receptors are involved in each stage of limb bud formation. In chicks, Wnt8c and Wnt2b (also known as Wnt13) in the lateral plate mesoderm activate the Wnt/β-catenin pathway and induce the expression of Fgf-10. Fgf-10 then induces Wnt3a, which in turn induces the expression of Fgf-8 *via* the Wnt/β-catenin pathway. These signaling events contribute to the formation of the apical ectodermal ridge (AER), which maintains the outgrowth of the limb bud. In addition, Wnt7a functions as a dorsal determinant of the limb bud. Because NRX is a negative regulator of the Wnt/β-catenin pathway and is expressed in the limb bud, it may be a modulator of limb-bud formation.

The involvement of ROS in the malformation of the limb bud has been suggested in several studies. Thalidomide is a known teratogen that induces phocomelia, a severe birth defect in which the arms are extremely shortened or absent (146). Parman *et al.* (134) reported that this effect can be explained by

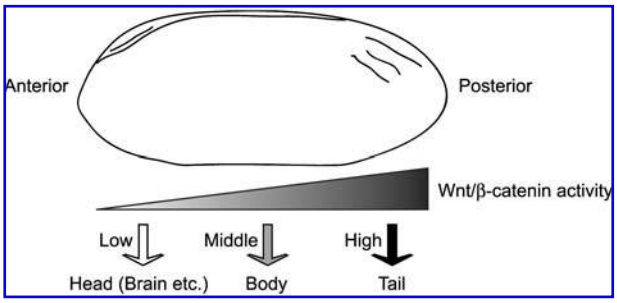

FIG. 11. Model of anterior–posterior patterning by the Wnt/β-catenin pathway. In *Xenopus* embryos, Wnt/β-catenin signaling is lower in the anterior region and higher in the posterior region. This gradient of Wnt/β-catenin signaling is thought to be important for body patterning, that is, the lower Wnt/β-catenin signaling in the anterior region is required for head formation, and the higher Wnt/β-catenin signaling in the posterior region is required for the body/tail formation.

the generation of ROS. Several mechanisms have been proposed and include ROS-induced DNA damage (134) and perturbation of the NF-κB pathway (44). Given that Wnt/β-catenin signaling is essential for normal limb-bud development, aberrant activation of the Wnt/β-catenin signaling induced by ROS *via* NRX may also contribute to limb-bud teratogenesis induced by thalidomide.

XI. INTERACTION OF NRX WITH PROTEIN PHOSPHATASE 2A (PP2A)

It was recently reported that NRX interacts with PP2A, a serine/threonine protein phosphatase that is involved in various processes such as cell-cycle regulation and tumorigenesis (93). PP2A is composed of a catalytic subunit $PP2A_C$, a structural subunit $PP2A_A$ (PR65), and one of the regulatory $PP2A_B$ subunits such as PR55 or B56. Lechward *et al.* (93) identified NRX as a protein that migrates at ~55 kDa (by SDS-PAGE) in PP2A preparations purified from rabbit skeletal muscle. They found that NRX interacts with purified $PP2A_C$ and with $PP2A_D$ (the dimeric form of $PP2A_C$ and $PP2A_A$). However, when they performed binding assays with $PP2A_{T55}$ (the trimeric form of $PP2A_C$, $PP2A_A$, and PR55), only $PP2A_C$ and $PP2A_A$ were bound to NRX; no PR55 signal was identified. These results led to the conclusion that NRX interacts with the dimeric form of $PP2A_C$ and $PP2A_A$ through direct binding to $PP2A_C$, thus displacing the third regulatory subunit. They then confirmed that ectopically expressed NRX binds to $PP2A_C$ and $PP2A_A$ in cells and that NRX immunoprecipitates possess significant phosphorylase phosphatase activity. This phosphatase activity is sensitive to low concentrations (*i.e.*, 5 n*M*) of the serine/threonine phosphatase inhibitor okadaic acid, supporting the notion that NRX interacts with PP2A in cells.

As described earlier, the ectopic expression of NRX results in the dephosphorylation of Dvl, but the mechanism remains uncharacterized. Considering the interaction between NRX and PP2A, an attractive possibility is that this dephosphorylation is mediated by PP2A recruited to Dvl *via* NRX. To our knowledge, no evidence indicates that the phosphorylation status of Dvl is regulated by PP2A. However, several reports have suggested the importance of PP2A in Wnt signaling. Seeling *et al.* (157) reported that B56, one of the $PP2A_B$ subunits, interacts with APC and that the ectopic expression of B56 decreases the amount of free β-catenin. In contrast, treatment of cells with okadaic acid, which inhibits PP2A, results in a significant increase in free β-catenin. The authors concluded that PP2A plays an inhibitory role in Wnt/β-catenin signaling through its phosphatase activity. The same group also reported that ectopic expression of not only B56 but also $PP2A_C$ and $PP2A_A$ in *Xenopus laevis* embryos results in a ventralized phenotype that reflects a decrease in Wnt/β-catenin signaling (101). These results clearly show that PP2A negatively regulates Wnt/β-catenin signaling. In contrast, Ratcliffe *et al.* (141) performed ectopic expression experiments in *Xenopus* and found that $PP2A_C$ expression enhances Wnt/β-catenin signaling. They found that this effect is independent of and occurs downstream of the stabilization of β-catenin. A loss-of-function analysis of B56 in *Xenopus* by another group showed a positive role of B56 in Wnt/β-catenin signaling (199). These inconsistent results make it difficult to describe the role of PP2A in Wnt/β-catenin signaling. It should be noted that many components of the Wnt/β-catenin signaling pathway are phosphoproteins that may be susceptible to dephosphorylation by different subsets of PP2A. Therefore, it should be emphasized that more careful studies are necessary to clarify the specific role of PP2A in Wnt/β-catenin signaling.

In the preceding section, we discussed the possibility that PP2A is involved in Wnt/β-catenin signaling through an interaction with NRX. Another possibility is that NRX regulates the activity of PP2A. Several protein tyrosine phosphatases (PTPs) have been shown to be regulated by reversible oxidation and reduction [reviewed by (17, 181, 197)]. PTPs contain a Cys residue in the catalytic center, and this is essential for the enzymatic reaction. This Cys residue is also prone to redox modification. In the case of PTP1B, the thiol group (-SH) of the Cys residue is oxidized to a sulfenyl group (-SOH), which then attacks the backbone nitrogen atom of an adjacent peptide bond (187). This results in the formation of a sulfenylamide group and the protection of the Cys residue from further oxidation to a sulfinyl group ($-SO_2H$) or a sulfonyl group ($-SO_3H$), both of which are not reduced by conventional intracellular reducing agents. The sulfenylamide form of PTP1B is enzymatically inactive because the catalytically essential Cys residue is inactive. However, when it is reduced, it becomes enzymatically active again, thus enabling the reversible regulation of phosphatase activity. Another example of reversible regulation is found in phosphatase and tensin homologue (PTEN), a dual phosphatase for tyrosine-phosphorylated proteins and phosphatidylinositol 3,4,5-trisphosphate (PIP_3). PTEN is also readily oxidized and inactivated, but it forms an intramolecular disulfide bond between the Cys residue at the catalytic center and a second Cys residue (95, 97). Oxidized PTEN can also be reduced and returned to an enzymatically active state. Indeed, the stimulation of cells with growth factors induces transient oxidation (inactivation) of PTEN followed by return to the reduced state (reactivation) (89). Such transient and reversible inactivation of PTEN may be important for the dynamic regulation of signaling molecules such as PIP_3. PP2A does not possess a catalytically essential Cys residue. However, a report suggests that the PP2A-C subunit is inactivated by oxidation, although the mechanism is unclear. When oxidized $PP2A_C$ is treated with reducing agents, it again becomes enzymatically active (35), suggesting the possibility that PP2A is also regulated in a redox-dependent and reversible manner. NRX may regulate the enzymatic activity of PP2A *via* its oxidoreductase activity.

Finally, the interaction between NRX and PP2A may be important for the regulation of PP2A, as is the case for Dvl. The addition of NRX to PP2A *in vitro* shows a very modest stimulatory effect on phosphatase activity [approximately twofold (93)], but this effect may be enhanced by unidentified intracellular molecules; NRX may recruit regulatory molecules to induce PP2A activity. If the interaction between NRX and PP2A is regulated by the redox state, PP2A activity may also be regulated in a manner similar to that of Dvl regulation. This is a speculative statement, and further research is required to confirm this.

XII. SUBCELLULAR LOCALIZATION OF NRX

One important issue that we should discuss is the subcellular localization of NRX. Kurooka *et al.* (88) first reported that overexpressed NRX in COS-7 cells is localized to the nucleus, whereas we observed that endogenous NRX in NIH3T3 localizes predominantly to the cytosol (39). These seemingly contradictory results may be explained by the assumption that NRX shuttles between the cytosol and nucleus. NRX does not appear to have any typical nuclear localization signal (NLS) or nuclear export signal (NES) sequences. Some reports suggest that Dvl and PP2A can shuttle between the cytosol and nucleus (69, 184). Therefore, one possible scheme to explain the nucleocytosolic shuttling of NRX is that it moves along with Dvl or PP2A as a complex. Dvl has been thought to be a cytosolic component of the Wnt/β-catenin signal. However, Itoh *et al.* (69) reported that Dvl also localizes to the nucleus, which is important for Wnt/β-catenin signal transduction (69). The precise mechanism underlying nuclear Dvl functions in Wnt/β-catenin signaling is still obscure. Further studies will reveal whether NRX can move into the nucleus as a complex with Dvl and whether it can affect Wnt/β-catenin signaling inside the nucleus. With regard to PP2A, it is known that this molecule can function in both the cytosol and nucleus (72, 163, 188). It will be interesting to examine whether NRX can affect these topologically different cellular events controlled by PP2A. Nevertheless, further research is required to reveal the detailed mechanism of the nucleocytosolic shuttling and location-dependent function of NRX.

XIII. CONCLUDING REMARKS

Research on NRX is in the early stages. However, much evidence indicates that NRX may play essential roles in numerous biologic phenomena. We have shown that NRX serves as a redox-sensor to regulate the Wnt/β-catenin signaling pathway. ROS are inevitable byproducts of cellular respiration and require detoxification, but cells also produce and harness ROS to control various cellular processes. It is interesting that signaling by Wnt/β-catenin, a well-established pathway for the control of cell proliferation and cell fate, also is involved in the utilization of ROS.

Many important points remain to be clarified. Studies conducted by Kurooka *et al.* (39), as well as those conducted in our laboratory, revealed that NRX possesses oxidoreductase activity (88). It is unknown whether such activity, observed in *in vitro* experiments, is physiologically important. The exact electron donors and the proteins or peptides that act as intracellular substrates also are unknown. The *cis*-Proline residue, which is implicated in the interaction of TRX family members with protein substrates, also appears to be conserved in NRX [Pro276 in human NRX (28, 63)]. Along with the existence of a PDI-b′-like domain in NRX, it seems that physiologic substrates for NRX are proteins or peptides or both, rather than other intracellular molecules such as nucleic acids and lipids. It is well known that the catalytic motif of the TRX family proteins is responsible for their redox potential and hence their physiologic functions. The catalytic motif of NRX is WCPPC, which differs from that of TRX (WCGPC). A report indicates that in the case of *Crithidia fasciculate* TryX2, the wild-type form (WCPPC) and its TRX-mimicking mutant form (WCGPC) showed similar reducing activity against insulin and TryX peroxidase (167). This result suggests that the WCPPC and WCGPC motifs do not have such a striking difference, at least in their reducing activities. Kurooka *et al.* (39) and we used DTT as an electron donor and assessed the oxidoreductase activity against insulin. The sequence of NRX is similar to that of TryX, and because no evidence shows the existence of trypanothione in mammals, the candidate for NRX electron donor may be the trypanothione-related peptide glutathione. We hope that the detail of the biochemical characterization of NRX and the components of the NRX oxidoreductase cascade will be elucidated in the future.

Experiments performed in our laboratory as well as studies by Zolnierowicz and colleagues (93) identified Dvl and PP2A as binding proteins for NRX (39). It is probable that other unidentified NRX-binding proteins exist. Our preliminary analyses indicate that this is the case (Takenaka *et al.,* unpublished observations). It is likely that NRX regulates the activity of not only Dvl and PP2A but also other participants in other signaling pathways.

Another unresolved issue is the role of NRX at the organism level. We have shown that NRX is a negative regulator of Wnt/β-catenin signaling in *Xenopus*. However, we do not know whether NRX acts as a redox-sensor of Wnt/β-catenin signaling inside living organisms. Clarification of this point will be critical for demonstrating the importance of redox signaling in organisms.

Myriad reports of ROS in the control of various biologic phenomena have been accumulating. However, at present, the precise molecular mechanisms involved are unclear. The concept of redox-dependent control of the Wnt/β-catenin pathway, in which ROS activity can be explained by the function of the protein NRX that possesses evolutionarily conserved ROS-reactive Cys residues and uses them for sensing intracellular redox conditions, is intriguing. Further examination of NRX will certainly contribute to the understanding of ROS signaling.

ABBREVIATIONS

AATF, apoptosis-antagonizing transcription factor; Abr, active breakpoint cluster region-related; ADL, adult T-cell leukemia–derived factor; AER, apical ectodermal ridge; APC, adenomatous polyposis coli; ASK1, apoptosis-regulated signal kinase 1; C9orf121, chromosome 9 open reading frame 121; CK, casein kinase; CREB, cyclic AMP response element–binding protein; DC1, divergent C1; DEP, Dvl, EGL-10, pleckstrin; DIX, Dvl, Axin; DTT, dithiothreitol; Dvl, Dishevelled; Dlg, Discs large; ER, endoplasmic reticulum; FADD, Fas-associated protein with death domain; FAP, familial adenomatous polyposis; Fgf, fibroblast growth factor; Frat, frequently rearranged in advanced T-cell lymphomas; Fzd, Frizzled; GBP, GSK3β-binding protein; GEMIN4, gem (nuclear organelle)-associated protein 4; GRX, glutaredoxin; GSK3β, glycogen synthase ki-

nase 3 beta; HCAP1, hepatocellular carcinoma–associated protein 1; HCCS1, hepatocellular carcinoma suppressor 1; Hh, hedgehog; HIC1, hypermethylated in cancer 1; HIF-1α, hypoxia-inducible factor-1α; H_2O_2, hydrogen peroxide; IP(3)R, inositol 1,4,5-triphosphate receptor; JNK, c-Jun N-terminal kinase; MAPKKK, mitogen-activated protein kinase kinase kinase; MnSOD, manganese superoxide dismutase; MO, morpholino oligonucleotide; MST, mammalian STE-20-like; NDP, nucleoside diphosphate; NF-κB, nuclear factor-kappa b; NIK, NF-κB inducing kinase; nNOS, neuronal nitric oxide synthase; NRX, nucleoredoxin; $O_2^{\cdot -}$, superoxide radical; $^{\cdot}OH$, hydroxyl radical; PAK, p21-activated kinase; PDI, protein disulfide isomerase; PDZ, PSD-95, Dlg, ZO1; PIP_3, phosphatidylinositol 3,4,5-triphosphate; PKA, protein kinase A; PMA, phorbol-12 myristate 13-acetate; PP, protein phosphatase; Prx, peroxiredoxin; PSD-95, postsynaptic density-95; PTEN, phosphatase and tensin homologue; PTP, protein tyrosine phosphatase; PX-12, 1-methylhydroxypropyl 2-imidazoloyl disulfide; RA, rheumatoid arthritis; RdCVF, rod-derived cone viability factor; Ref-1, redox factor-1; RNAi, RNA interference; Rnmtl1, RNA methyltransferase–like 1; ROS, reactive oxygen species; sFRP, secreted Fzd related protein; SpTRX, sperm-specific TRX; TCF/LEF, T-cell factor/lymphoid enhancer factor; TGF-β, transforming growth factor β; Timm22, tranlocase of inner mitochondrial membrane 22; TNF-α, tumor necrosis factor-α; TR, trypanothione reductase; TRP14, TRX-related protein 14; TRP32, TRX-related protein 32; TMX, TRX-related transmembrane protein; TRX, thioredoxin; TrxR, TRX reductase; TryX, tryparedoxin; VEGF, vascular endothelial growth factor; Wnt, wingless related; ZO1, zona occludens 1.

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Date of first submission to ARS Central, December 26, 2006; date of final revised submission, April 5, 2007; date of acceptance, April 7, 2007.

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