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Cellular mechanotransduction of physical force and organ response to exercise-induced mechanical stimuli

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Abstract The variable effects of mechanical stimuli induced by exercise on the human body are becoming better understood. Additionally, the indispensable effect of constant gravity on the human body to construct and maintain living organisms is known from observed muscle and bone regression induced by long-term recumbency or visits to gravity-free environments, such as space. Based on reactions of the body, cells faced with various inputs largely depend on gene expression, biochemical processes, or both. Thus, it is easy to imagine that physical input can be converted into a chemical process. The conversion process that changes physical forces (mechanical stimuli) into chemical reactions in a cell is called mechanotransduction. A growing number of studies examining mechanotransduction have led to a new phase in the understanding of exercise-induced effects on organisms.

Keywords : mechanostimuli, mechanosensors, signal processing, human body

Introduction

Exercise can offer prominent favorable effects, such as increased bone mass, muscle hypertrophy[1,2], and improved cardiovascular system function[3,4]. Physical force is understood as the primary cause of these effects. However, because the final outputs of organs are changes in the expression levels of genes or proteins, exercise-induced physical forces must be converted into biochemical stimuli. This ability to sense cellular force and convert it into biochemical stimuli is called mechanotransduction. However, only a small number of molecules have been experimentally detected that are capable of sensing and transducing mechanostimuli, for example, stress-activated cation channels (SACs)[5-7] and cytoskeleton-related proteins[8,9]. One molecule, mammalian target of rapamycin (mTOR), has long been known as a regulator of protein synthesis; but it is currently attracting attention for its mechanoreactivity[10,11]. The nomenclature for this molecule has now changed to "mammalian/mechanistic target of rapamycin," reflecting the rising recognition of its critical role in mechanotransduction. However, even mTOR works downstream of the conversion of mechanostimuli to chemical reactions, and the molecular mechanisms of the conversion remain an enigma.

Historically, biochemical technologies have greatly contributed to the improvement of intracellular signal transduction research. Solubilization is a basic method in biochemistry, and this technology proved powerful in the analysis of cellular soluble fractions in the early days of signal transduction research. However, cells have a considerable amount of insoluble substances, a large portion of which are part of the cytoskeleton or nuclei, making this subcellular fraction beyond the scope of early biochemical research. Moreover, mechanotransduction research has traditionally required multidisciplinary cell biological technologies because of the dynamic nature of the cellular mechanism, and this dynamic nature doesn't fit well with biochemical measurements for analyses of sedentary samples prepared by solubilization. Thus, the discovery and recognition of signal transduction in soluble fractions preceded that of mechanotransduction. However, the potential of this research field remains an unexplored frontier, ripe with the possibility of contributing to future biological and medical knowledge.

This review summarizes some of the effects of exercise on body organs and also reviews current knowledge regarding mechanotransduction, focusing on the molecular basis of this mechanism. Finally, a model is proposed that connects the mechanosensing of molecules with the biological response generated by the organ perceiving the mechanical stimuli.

Effects of exercise on human organs

Exercise can be described as "potent medicine" that improves many aspects of an individual's health[10]. Aerobic exercise greatly contributes to fat metabolism by consuming fat as an energy supply, and muscle hypertrophy stimulated by anaerobic exercise can enhance the basic

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metabolic rate. Thus, a combination of aerobic and anaerobic exercises may effectively prevent obesity, hyperglycemia, hypertension, and hyperlipidemia. The effects of exercise lead to increases in muscle and bone mass[1,2,10,11] and flexibility of blood vessels[3,4], mediated not only by the upregulation of protein synthesis[10,11] and bone metabolism[12,13], but also by cell and tissue proliferation and differentiation[12-14]. Different modes of exercise generate different effects. Thus, eccentric and concentric contractions affect muscles differently, that is, slow-twitch muscle fibers exhibit a lower threshold for tension than fast-twitch muscle, a concept known as the "size principle"; however, fast-twitch muscle under eccentric contraction does not conform to this principle[15,16]. Therefore, load resistance/strength exercise with eccentric contractions is useful for effective training of fast-twitch muscle.

The participation of mTOR in the exercise-induced hypertrophy of skeletal muscle is well documented. One of the atypical serine/threonine kinases, mTOR exists in two distinct protein complexes called mTORC1 and mTORC2, which have different sensitivities to rapamycin and use different signaling pathways. The mTORC1 complex regulates mRNA translation to protein as well as lipid synthesis mediated by direct phosphorylation of $p70^{S6k}$, autophagy, and HIF-1-mediated energy metabolism independent of $p70^{S6k}$ phosphorylation; while mTORC2 participates in cell survival as well as cytoskeletal reorganization[17]. Exercise-induced phosphorylation of $p70^{S6K}$ led to the discovery that mTOR contributes to muscle hypertrophy[18]. Because rapamycin strongly inhibited this phosphorylation[19], mTOR was suggested to participate in this process. Mechanical stimulation was found to upregulate protein synthesis in muscle, and the initiation of that translation was found to be a major regulation point[20,21]. The binding of mRNAs bearing 5'-initiation of translation (TOP) structure to polysomes is enhanced by mechanical stimuli[22]. A 5'-TOP structure consists of a 7-methylguanosine cap attached to the 5' end of the mRNA followed by a polypyrimidine tract, and is frequently found on mRNAs encoding proteins that are closely related to cell growth, such as ribosomal proteins and translation regulating factors[23]. The increase in the translation of these genes induced by mechanotransduction suggests a close relationship between mechanostimulation and muscle hypertrophy. Rapamycin selectively reduces translation of mRNA for these genes. The direct inhibition of $p70^{S6K}$ by S6K inhibitors does not suppress the translation of these genes, suggesting that the mTOR molecule is essential to mechanostimulus-induced muscle hypertrophy in addition to S6K-mediated signaling[24].

Another mechanism for mechanostimulus-induced muscle hypertrophy, recently found to be important, is mediated through miRNA regulation. A growing body of evidence indicates that mechanisms mediated by RNA interference regulate a broad range of transcription products, and the conventional concept of "the gene" is no longer sufficient to appropriately describe these genetic events. The international research consortium FANTOM project (Functional Annotation of Mammalian Genome; http://www.osc.riken.jp), executed at the initiative of Hayashizaki et al., discovered a tremendous number of non-protein-coding RNAs that are transcribed[25,26]. Conventional genes occupied only 2% of the genome, while their new concept led to the finding that 70% of the genome was transcribed and participated in the regulation of whole mRNA in the cell. A group of miRNAs called myomiR (miR-1a-1, miR-1a-2, miR-1331-1, miR-133a-2, 133b, miR-206, miR-208a, miR-208b, miR-486, miR-499) is specifically expressed in the muscle[27]. Interestingly, there are heart muscle-specific (miR-208a) and skeletal muscle-specific species, especially slow-twitch muscle-specific species (miR-206, -208b, -499); however, no fast-twitch muscle-specific miRNA has been found. For non-muscle-specific miRNA, miR-196a and miR-885 exhibit higher expression in fast-twitch muscles than in slow-twitch muscles.

Although, myomiR knockout mice exhibit no significant phenotypes in many cases[27], some implications for the biological roles of myomiR on stress responses have been suggested, such as the delay of recovery from denervation[28] and its requirement in stress-induced heart muscle hypertrophy[29]. Despite the immaturity of this research field, several fascinating hypotheses on the regulation of muscles have been presented[27]. Thus, it has been suggested that miR-1 and miR-133a negatively regulate the IGF-1/Akt signaling cascade. The downregulation of these two miRNAs during acute resistance exercise leads to an enhanced IGF-1/Akt signal that culminates in protein synthesis. It has also been suggested that miR-378 inhibits one of the transcription repressors, MyoR, which suppresses MyoD expression as well as one of the nuclear receptors, peroxisome proliferator-activated receptor γ coactivator-1β (PGC-1β). During the chronic phase of resistance exercise, miR-378 is downregulated in low-response muscle fibers, which is accompanied by mitochondria synthesis as a consequence of PGC-1β upregulation. In high-response muscle, retained expression of miR-378 holds MyoD expression high, which leads to muscle differentiation that culminates in muscle hypertrophy.

During endurance exercise, the downregulation of miR-23, miR-494, and miR-16 is observed in skeletal muscle. Because miR-23 and miR-494 prevent the expression of genes that participate in mitochondria synthesis, such as PGC-1α, while miR-16 inhibits the expression of vascular endothelial growth factor and its receptor, downregulation of these miRNAs may contribute to the mitochondria synthesis and angiogenesis induced by endurance exercise. The circulating level of miR-486 is downregulated during endurance exercise, both in the acute and chronic phases. This downregulation can enhance the expression of miR-486 target genes, such as the forkhead transcription factor (FOXO) and phosphatase and tensin homolog deleted on

chromosome 10 (PTEN), and may function to upregulate protein turnover. Endurance exercise upregulates miR-29 expression in heart muscle. A suppressive effect of miR-29 on collagen gene expression may contribute to avoiding fibrosis in heart muscle fibers and retaining heart ventricular compliance.

Recent significant progress has been achieved in understanding the mechanostimulus processing mechanism in bone. Bone functions not only to underpin the structure of individuals, but also as the storage of calcium ions. The remodeling of bones is executed by repeated osteogenesis and bone resorption via osteoblasts and osteoclasts, respectively. The importance of mechanostimuli for bone remodeling is recognized and described in "Wolff's law," which maintains that bone will adapt to the loads under which it is placed, becoming weaker if the load decreases[30]. The osteocyte embedded in the intra-bone cavity perceives the load on the bones while maintaining connections with other osteocytes mediated by thin cellular processes[31]. Receptor activator of nuclear factor kappa-B ligand (RANKL) has been shown to act as a differentiation factor for the osteoclast by stimulating precursor cells. The cellular source of RANKL has been discovered. Takayanagi et al. succeeded in establishing a method for the isolation and pure culture of osteocytes, and found high expression of RANKL in the osteoclast. RANKL gene knockout mice with osteocytes that exhibit osteopetrosis suggested osteocytes as the source of RANKL. Moreover, the osteopetrosis was not observed in neonatal knockout mice, but became extensive during the growth of the mice, suggesting a critical role for mechanical stimulation processed by osteocytes for appropriate RANKL levels to develop normal osteogenesis and bone remodeling[32].

Endothelial cells (ECs) constantly perceive mechanical stress from the blood stream. This shear stress stimulation regulates many cellular events in blood vessels, such as the regulation of morphology and the release of nitric oxide (NO), prostacyclins, C-type natriuretic peptide, adrenomedullin, thrombomodulin, platelet-derived growth factor, and transforming growth factor. The majority of these events are controlled during the regulation of gene expression, such as during transcription (responsive DNA elements have been identified) and the stabilization of mRNA. The transduction of shear stress is mediated by calcium ion (Ca^{2+}) influx, and the amount of this influx is regulated by the strength of the stress[33]. Rates and amounts of shear stress can be modified by changing the viscosity of the media. Quantitative studies examining shear stress revealed that Ca^{2+} influx is dependent not on flow rate but on the amount of shear stress[33]. This fact suggests that ECs perceive the change in shear stress induced by a change in blood flow and translate this into a change in Ca^{2+} influx. As changes in blood flow can be induced by exercise and perceived by ECs, the following described responses and regulations are plausible. High shear stress induces downregulation in the secretion of endothelin-1, which is a potent vasopressor substance, and upregulation in NO production from ECs[34]. The difference in the levels of circulating endothelin-1 is increased in non-working muscles during exercise, while the difference is unchanged in working muscles, implying an increase of endothelin-1 consumption in non-working muscles. Simultaneously, exercise increases endothelin-1 production in the kidney. It is plausible that this series of reactions to exercise downregulates blood flow to the non-working muscles and internal organs to reserve a sufficient blood supply for the working muscles[35]. Circulating endothelin-1 increases with age[36,37], whereas endurance/aerobic exercise induces downregulation of circulating endothelin-1 levels in the resting phase, even in the aged[38]. Although exercise affects NO production, the exact effects remain controversial. Various reports indicate that an upregulation of NO production in working muscle contributes to blood flow increases as well as decreases, or that recruitment of NO occurs after exercise. Higher resting levels of circulating NO have been observed in people who habitually participate in endurance exercise than in those who do not, and upregulation of endothelial NOS mRNA accompanied by exercise has also been proposed[39,40].

Thus, exercise changes in the cellular, tissue, or organ phenotype have been widely established and accepted based on data derived from both empirical observations in humans and experimental studies in humans and animals. However, the mystery that remains unsolved is the mechanism whereby exercise culminates in the effects described above, and this mystery is arguably more puzzling than the mechanisms of cell growth control or oncogenesis. One reason for the delay in elucidating this mechanism may be that the mechanisms for the conversion of physical stimuli, such as exercise, into biological/chemical reactions, such as biochemical or gene expression reactions, are not yet fully elucidated. However, progress in studies on mTOR may help elucidate these mechanisms[10,11]. The pathway of IGF-1 → phosphatidylinositol 3-Kinase (PI3K) → protein kinase B (PKB)/Akt → mTOR may participate in the recruitment of mTOR activation for the transduction of exercise effects[41-43]. However, exercise-induced muscle hypertrophy was observed even in transgenic mice expressing the dominant negative form of IGF-1 in muscle[44]. In addition, the results of experiments conducted using PI3K inhibitors and/or PKB/Akt knockout mice showed that the above described signaling cascade, while adequate, is not essential for recruiting mTOR activity[45-47]. Whether stress-activated channels can generate an mTOR activation signal upon perception of mechanostimuli is not well studied, and the results obtained to date are controversial[48,49]. A promising suggestion is that mechanostress induces an increase in cellular phosphatidic acid (PA) levels[47,49,50]. Phospholipase D (PLD) is an enzyme implicated much more than phospholipase C

(PLC) as a promising candidate responsible for this reaction[49]. PA binds directly to and activates mTOR[51,52], and may act as a mediator that transduces physical stimuli information into mTOR activation. This PA-mediated pathway is independent of the pathway mediated by PI3K, as mentioned above[47], and was expected to be the main pathway from mechanostimuli to mTOR activation. However, since PLD was also found to be insufficient for the upregulation of the entire amount of PA induced by mechanostimuli[49], understanding the mechanism for the conversion of mechanostimuli to a chemical reaction will require further elucidation.

The next section summarizes the current understanding of mechanosensing molecules.

What is mechanotransduction?

In 1997, Ingber's group suggested the innovative concept that an intracellular component existed that perceived cellular survival or growth without the aid of soluble factors[53]. They conducted a cell culture experiment using a culture dish that contained regions treated to allow for variably-sized areas of cell adhesion within a single dish, and found the existence of an area threshold required for cell survival and growth, as well as a clear dependency on the size of the cell adhesion area for rates of survival and growth. The culture conditions for all cells were the same because the experiment was performed within a single undivided dish. Thus, since the components contained in the cell culture medium were common to all cells, the area was undoubtedly the only variable, and the authors concluded that the cytoskeleton was a promising candidate as the intracellular component that perceives area information. Their observations also suggested that once a physical force given to a cell is perceived by the cytoskeleton or related components, it can be translated into biochemical reactions. From their analyses, they generated the concept of "tensegrity" (tension + integrity), which suggests that cells regulate their functions by interacting with tension[54]. The next step was to determine which molecular substances have the ability to recognize tensegrity.

Although many reports offer candidate molecules thought to participate in mechanosensing, unexpectedly, few molecules are supported by the experimental evidence. Those are summarized in Table 1 and Fig. 1. SACs have the longest research history in this field. The patch clamp method overcame the aforementioned biochemical problem of solubility and was used to establish a change in the activity of the channels induced by mechanical stimuli. Many reports suggested SACs as candidate mechanosensors from the observations that channel activities are altered during mechanosensing. However, the molecules showing activity changes in which mechanostress-induced structural change has been confirmed

Table 1. Mechanosensor molecules

Category	Name	Distributions	Function	References
Stress-Activated Channels	TRPC subfamily	Cardiovascular organs, Nociceptors	Nociception	67) Ma et al. (2010)
	TRPV subfamily	Nociceptors, Neurons	Nociception	68) Sokabe et al. (2010)
	TRPM subfamily	Various	Shear stress perception	69) Wei et al. (2009)
	TRPP1, TRPP2	Cardiovascular organs	Shear stress perception	70) Shalif-Naeini et al. (2009)
Cytoskeleton Related Proteins	p130Cas	Membrane skeleton	Tension perception	8) Sawada et al. (2006)
	Talin	Focal adhesions	Tension perception	9) del Rio et al. (2009)

Each TRP except TRPP1/2 contains several members of the subfamily. References listed are representative ones among the subfamily for TRPs.

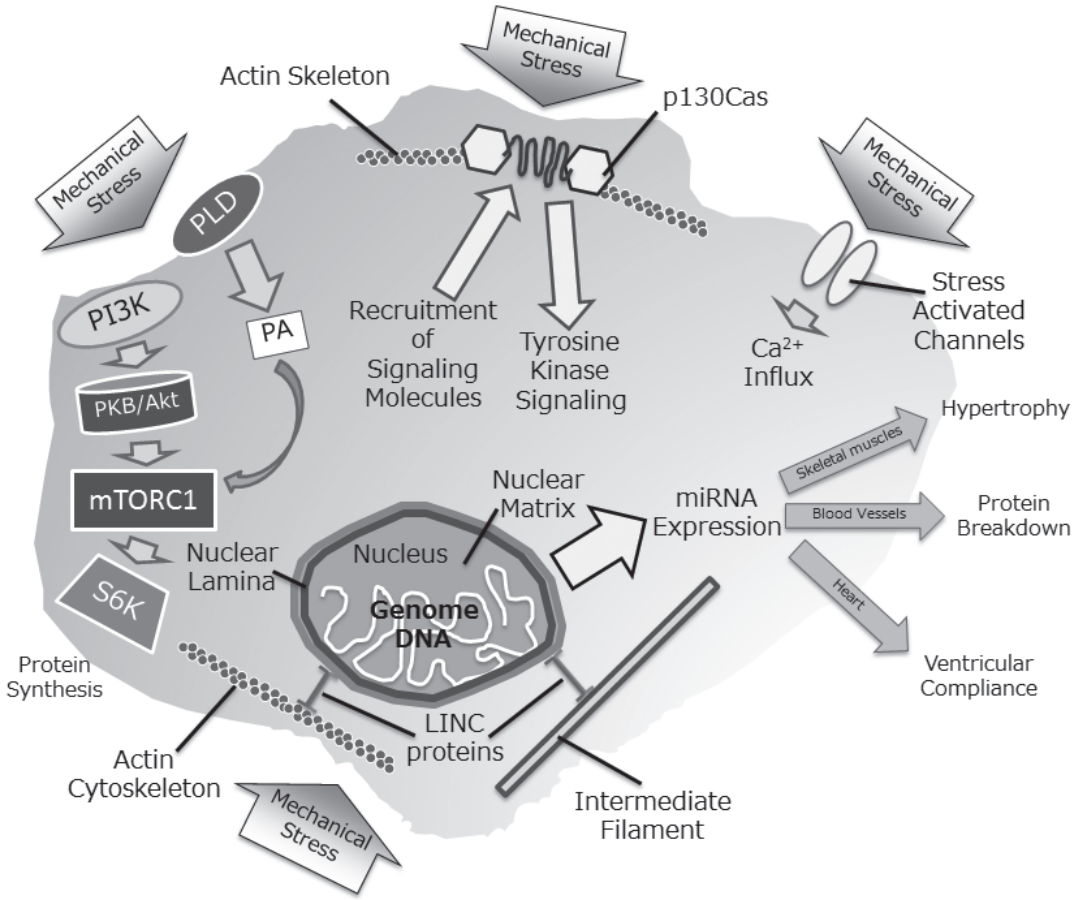

Fig. 1 Mechanosensing systems and cellular responses induced by mechanostress.
Four mechanosensing systems and gene/protein expression regulation are summarized. PLD: phospholipase D, PI3K: phosphatidylinositol 3 kinase, PA: phosphatidic acid, PKB/Akt: protein kinase B/Akt, S6K: $p70^{S6K}$, LINC: linker of nucleoskeleton and cytoskeleton complex.

are limited to bacterial molecules, leaving open the debate about whether SACs are bona fide mechanosensor molecules. Candidate mechanosensor SACs in eukaryotes are members of the transient receptor potential (TRP) channel family. Among the six TRPs - TRPC ("C" for canonical), TRPM ("M" for melastatin), TRPV ("V" for vanilloid), TRPML ("ML" for mucolipin), TRPA ("A" for ankyrin), and TRPP ("P" for polycystic) - TRPC, TRPM, TRPV, and TRPP are reportedly correlated with mechanotransduction[55]. The TRP channels linked with the cytoskeleton open upon perception of a mechanical force and exhibit altered channel activity[55]. An interesting correlation between TRP and mTOR has been presented[56]. A mechanical load induces neuronal nitric oxide synthase-mediated NO production in skeletal muscle and subsequent peroxynitrite ($ONOO^-$) production by the oxygen radical producing enzyme nicotinamide adenine dinucleotide phosphate-oxidase 4 (NADPH oxidase 4 or Nox4) as well as mTOR activation. This mTOR activation was thought to be induced by recruiting Ca^{2+} through $ONOO^-$ stimulation of TRPV1. The mTOR activation as well as muscle hypertrophy was confirmed by stimulating TRPV1 with its ligand capsaicin in the absence of exercise[56]. However, even in this elegant analysis, the initial conversion of mechanical stimuli into NO production is still an enigma, and the mechanosensing and converting molecule/machinery remains unknown.

The best-documented molecules for their participation in the generation of chemical reactions from mechanostimuli as their structures change are p130Cas and talin[8,9,57,58]. The discovery of p130Cas as a mechanosensor was uniquely achieved. This molecule was already well known as one of the integrin assembling proteins that accepts tyrosine phosphorylation[59] and for its function to upregulate cellular motility opposing paxillin, a well-known tyrosine phosphorylation acceptor integrin assembly protein[60]. The p130Cas molecule is characterized by 15 repeats of tyrosine phosphorylation acceptor motifs in the central substrate domain and by its binding to membrane-cytoskeletal fractions at its N- and C-termini. Sawada et al. screened cytoskeletal as well as cytoskeleton-related proteins that exhibited alterations in the protein phosphorylation cascade by conformational changes induced by loading stretch stimuli onto the Triton-insoluble cytoskeletal fraction[8]. They determined that tyrosine phosphorylated p130Cas is a molecule that perceives membrane stretch. The phosphorylation is cell stretch-dependent and is required for the activation of

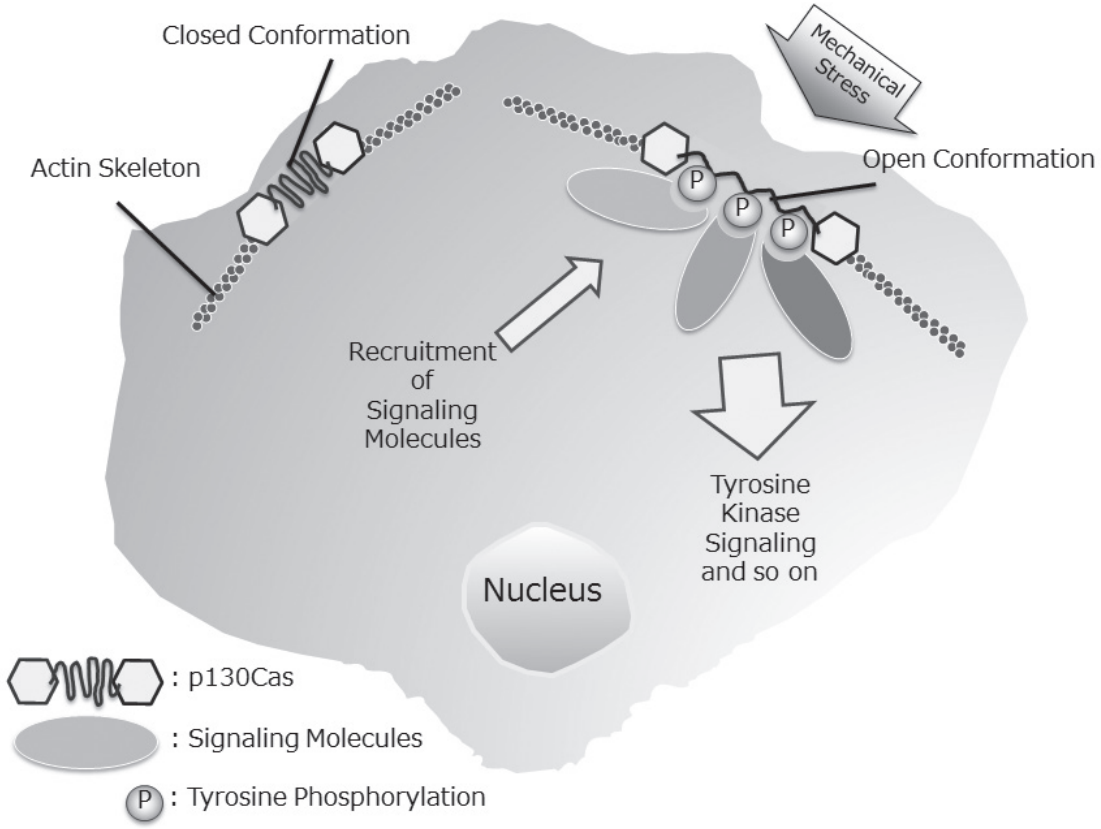

Fig. 2 Stretch sensing of plasma membrane-skeleton by p130Cas.
The closed conformation of p130Cas is presented on the left side of the scheme, and the open conformation is drawn on the right. Signaling molecules downstream of the mechanostress are recruited to the p130Cas in the open conformation during the stretching of the plasma membrane to convert mechanical stimuli into biochemical processes. Tyrosine phosphorylation of the central p130Cas substrate domain is thought to act in the recruitment of signaling molecules.

one of the downstream GTP-binding proteins Rap1[8]. The open conformation of p130Cas was thought to occur by cell stretching in correlation with the anchorage of both N- and C-termini of p130Cas in the cytoskeleton. This led to the sensitization of p130Cas to Src family kinase-mediated phosphorylation, as well as to the generation of downstream signals. Sawada et al. also found several binding proteins that bound to the stretched cytoskeleton simultaneously with p130Cas, including focal adhesion kinase (FAK), paxillin, and PKB/Akt[61]. FAK acts as a tyrosine kinase to various downstream factors, as well as a scaffold protein to assemble many signaling proteins in submembranous regions[62]. Although paxillin itself has no enzymatic activity, the molecule acts as a scaffold, similar to p130Cas and FAK, and can recruit Src homology region 2 motif-bearing proteins, such as p120 RasGAP, the p85 PI3K subunit, and/or the adaptor molecule crk, by directly binding to phosphorylated tyrosine residues, leading to the generation of cell adhesion signals[63]. For example, paxillin can generate local suppression of Rho activity at paxillin-localized submembranous areas by recruiting p190 RhoGAP, which is mediated by direct binding to the p120 RasGAP that is bound to tyrosine phosphorylated paxillin[63]. The perception of membrane stretching by the cytoskeleton and the conversion to a phosphorylation signal by p130Cas, together with other scaffold proteins as well as kinases that can generate a broad range of downstream signals, are promising findings for the understanding of a variety of cellular responses induced by mechanical stimuli.

A possible mechanostimulus processing mechanism

The results from Sawada et al. suggested the generation of cellular stretch-specific intracellular signals and informed the hypotheses outlined below (Fig. 2).

A low dose of eccentric contraction induces fast-twitch skeletal muscle contraction disagreement according to "the size principle." This phenomenon suggests sensitization of the fast-twitch muscle induced by eccentric contraction. The p130Cas molecule is in open conformation upon muscle stretch and can be tyrosine phosphorylated. Subsequently, the phosphorylated p130Cas recruits various signaling molecules to the subplasma membranous membrane-skeleton fraction, culminating in the reduction of the threshold for generation downstream signaling. The subplasma membranous recruitment of Src or FAK and the increased rate of contact with substrates are thought to

be a plausible mechanism for this activation, and, indeed, this signaling cascade governs focal adhesion formation. Thus, p130Cas is a model of a molecule that may be specifically recruited during plasma membrane stretch and that may lead to converted output as alterations of enzymatic activities.

Additionally, other than the p130Cas subplasma membranous conversion model described above, direct transduction of mechanical force to the nucleus is also suggested[64-66] (Fig. 1), although discussion of this mechanism is limited by the scope of this review. Briefly, the linker of nucleoskeleton and cytoskeleton complexes localizes between the cytoskeleton and the nucleus, and thus may be the entity directly transducing the mechanical force perceived by the cytoskeleton toward the nucleus. The transduced force may modulate various epigenetic regulations, such as chromatin remodeling mediated by nuclear lamina or nuclear matrices. However, these mechanisms are less well established than the aforementioned transduction mechanisms and await further exploration.

Concluding remarks

Mechanotransduction research is at an exciting frontier. The importance of the roles that mechanotransduction plays is beyond providing structure to multicellular organisms. Our understanding of this field to date has been supported by biological analyses. One of the translational potentials of this field is the proposal of "mechanopharmacology," which attempts to evaluate the effects of mechanostimuli by quantitation and translation into a pharmacological dose, and to analyze the correlations aimed at drug discovery. Since muscles, bones, and blood vessels are under regulations mediated by mechanotransduction, and also are intimately correlated with an individual's health, research contributions from this field will improve future therapeutic options.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

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